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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

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BIOLOGICAL BULLETIN

THE BREATHING AND FEEDING MECHANISM OF THE LAMPREYS.[1]

JEAN DAWSON.

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The present study of the respiratory mechanism of the lampreys was undertaken at the suggestion of Professor Jacob Reighard and has been carried out under his direction. Preserved material of *Petromyzon marinus* Linnæus, *Petromyzon marinus unicolor* (De Kay) and *Lampetra wilderi* Gage has been examined, but these three species have been found to be so nearly alike in the structure of their respiratory mechanism that the following anatomical description of *Petromyzon marinus* holds good in all essentials for the other two species. Where measurements are given they are taken from *P. marinus* and refer to individuals of average size. The close relation between feeding and breathing in the lamprey has made it necessary to consider the mechanisms of the two together. There is without doubt a similar close relation between the mechanism of respiration and that of circulation but this has not been included in the scope of the present paper.

I. STRUCTURES INVOLVED IN BREATHING AND FEEDING.

Food entering the funnel-shaped cavity of the oral hood passes thence at the apex of the oral hood into the mouth cavity

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[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 92.

(Fig. 1, *b*). From the mouth cavity it passes through a constriction into the larger so-called pharyngeal cavity (*d*). From the pharyngeal cavity food enters the alimentary canal proper by way of the slender œsophagus (*p*) while water may conceivably enter the much larger water tube (*s*). The latter lies ventral to the œsophagus, extends caudad to the pericardial cavity, and there ends blind. Should water enter it from the pharynx it could then pour through the seven openings on each side into the gill sacs, from which it could reach the exterior by the seven external branchial openings on each side of the body (Fig. 5, *h*).

The opening from the pharynx into the water tube is guarded by a pair of velar valves (Fig. 1, *l*; Fig. 13, *l*), while valves guard also the external branchial openings (Fig. 13, *c*, *b*). Muscles control the opening between mouth cavity and pharynx and between pharynx and œsophagus. In order to understand the mechanism of these parts, each must be considered in greater detail.

A. *Oral Funnel, Mouth Cavity and Tongue.* — The oral funnel opens upon the ventral side of the anterior part of the head. In the specimens examined the average diameter of its external opening is 3.5 cm., while its dorsal opening, where it becomes continuous with the mouth cavity, has a diameter of 1 cm. Around the smaller opening of the funnel, on the boundary between it and the mouth cavity, is a supporting ring of cartilage, called the annular cartilage (Fig. 1, *t*). Attached to this cartilage and forming the muscular walls of the funnel is a three-layered muscle, the annularis (Fig 1, *u*). Fürbringer (1875) reports that the outer and middle layers of the annularis serve to attach the animal by the oral funnel while the inner layer causes the walls of the funnel to expand and contract, and this, together with the simultaneous closing of the mouth opening (opening between the oral funnel and mouth cavity), gives the animal power to suck blood or tissue which it has succeeded in rasping from its prey. The walls of the funnel become thin abruptly at their external edge, and there bear a thick fringe of rather short tentacles (Figs. 1, *i*; Fig. 2, *i*).

The posterior wall of the funnel forms with the ventral wall of the mouth cavity an angle of almost 90 degrees; the anterior

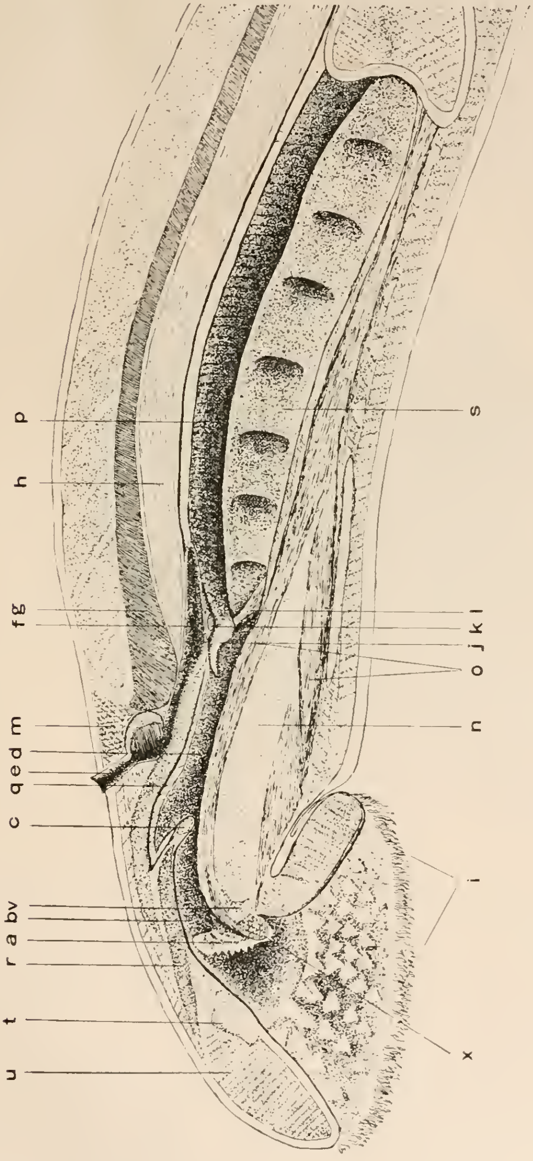

FIG. 1. Median longitudinal section through the head of *P. marinus.* *a*, tongue; *b*, mouth cavity; *c*, semiannularis muscle; *d*, pharynx; *e*, nasal tube; *f*, nasal cœcum; *g*, brain; *h*, notochord; *i*, buccal fringe; *j*, velar jaw; *k*, posterior pharyngeus muscle; *l*, velar valve; *m*, connective tissue surrounding the epiphysis; *n*, lingual cartilage; *o*, muscles of tongue; *p*, œsophagus; *q*, ethmoid cartilage; *r*, semiannularis cartilage; *s*, water tube; *t*, annular cartilage; *u*, annularis muscle; *v*, central cartilage of the tongue; *x*, anterior lobe of the tongue.

wall meets the dorsal walls of the mouth in a much gentler curve.

The funnel is lined with thick mucous membrane bearing sharp conical teeth (Fig. 2). The teeth are arranged in a series of concentric loops, the crossed ends of which lie in the anterior portion of the funnel (Figs. 1 and 2). The result of this arrangement is that any radius drawn from the center of the mouth opening strikes at least two teeth except over the posterior one fourth of the circumference of the funnel. This posterior one fourth of the funnel has teeth approximately on radii running from that portion on the central loop which has an arc of the smallest radius. This is a necessary result of the geometric relation of the concentric loops (Figs. 2 and 3). The teeth on the central loop are so crowded that there are as many as on the larger outer loop. These teeth are also larger and are grown together in plates of two and more.

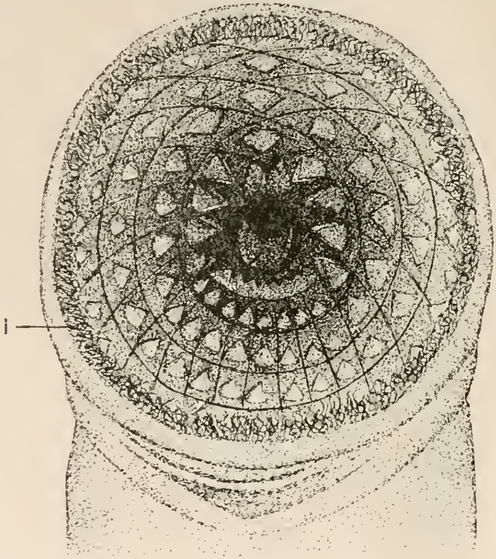

Fig. 2. Mouth of *P. marinus* showing the arrangement of the teeth in concentric loops.

The end of the tongue (Fig. 1, Fig. 5 and Fig. 6) has two lateral lobes (*a*) which are covered with smooth mucous membrane and an anterior lobe (*x*) covered with plates of sharp teeth. This free end of the tongue may be seen projecting for 1 or 2 mm. into the caudal end of the funnel and the teeth on its anterior lobe are thus brought into the same plane as the central teeth of the funnel and function with them in rasping. The great freedom of movement of the end of the tongue necessary in rasping is attained by its central cartilage (Fig. 1, *v*) being attached by a sort of socket joint to the enlarged anterior end of the large lingual cartilage (Fig. 1, *n*).

The mouth cavity is short and curved, about five times as long as it is wide at its middle, and extends from the oral funnel to the pharynx, a distance of about 2 cm. Its anterior end, where it meets the funnel, is larger than in any other part. Its roof and sides are dome shaped and are formed by the semiannularis cartilage in front and by the semiannularis muscle behind (Fig. 1, *r*, *c*). The muscle arises from the posterior border of the cartilage and forms a very small portion of the roof of the mouth. The posterior portion of the dome formed by the semiannularis muscle arches so close to the ventral wall of the mouth that it greatly constricts the cavity in this region and when it contracts, completely shuts off the mouth cavity from the pharynx. The floor

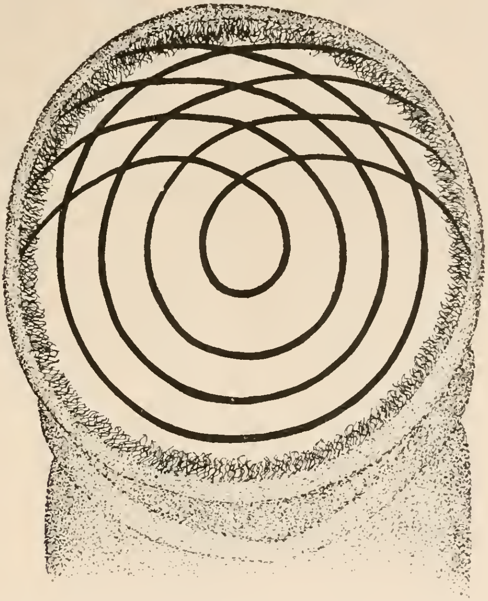

FIG. 3. Diagram of the arrangement of the teeth in the oral funnel of *P. marinus*. The heavy lines show the loops upon which the teeth are inserted.

of the mouth is formed by the large underlying tongue muscle (Fig. 1, *o*). The tongue as a whole is a large, long cone which stretches from the mouth opening to the ventral anterior portion of the pericardium to which it is attached by its apex. Thus the tongue underlies the whole mouth cavity, pharynx, and water tube. When the tongue is pushed forward so that its lobes occupy the flaring anterior part of the mouth cavity, its lateral lobes fall apart (Figs. 5, 6) and there is left between them a passage-way which connects the cavity of the oral funnel and that of the mouth. This passage-way, which then has a cross section equal to that of the mouth cavity at its middle, diminishes rapidly in diameter as the tongue is drawn back into the narrower part of the mouth cavity until at last it is completely closed before the tongue has reached the posterior end of the semiannularis cartilage. The end of the tongue is thus seen to act as a piston working with a short stroke back and forth in the mouth cavity, the walls of which form the cylinder while the free end of the tongue forms the piston-head and its lateral lobes the valves.

B. *Pharynx.* — Passing out of the mouth cavity through the small opening ventral to the semiannularis muscle, the food enters the cavity of the pharynx. This cavity is somewhat irregular (Fig. 1, *d*). Its dorsal anterior portion is wedge-shaped in longitudinal section and extends forward over the semiannularis muscle and semiannularis cartilage. This portion of the pharyngeal cavity thus lies dorsal to the posterior portion of the mouth cavity. The remainder of the pharyngeal cavity is about 2.5 cm. in length from where it joins the mouth cavity to where it opens into the œsophagus and water tube. Instead of having a wall whose mucous membrane lies directly upon the tongue muscles on the ventral side and against the cartilage on the dorsal side, as in the case of the mouth cavity, there is, in the wall of the pharyngeal cavity, a thin layer of muscular tissue, the pharyngeus muscle intervening between the mucous membrane and the outer layers of muscle or cartilage. The pharyngeus (Fig. 4, *c*), which is fully described by Fürbringer (1875), entirely envelops the pharyngeal cavity and ends on a raphe on the mid-ventral line of the cavity. It is in contact with the mucous membrane except in the ventral lateral part of the pharynx. Here between the pharyngeus muscle and the mucous lining of the cavity on its lateral walls are stretched two muscles, the hyomandibulari-semiannularis and the hyomandibulari-glossus. These muscles cause the mucous membrane to project

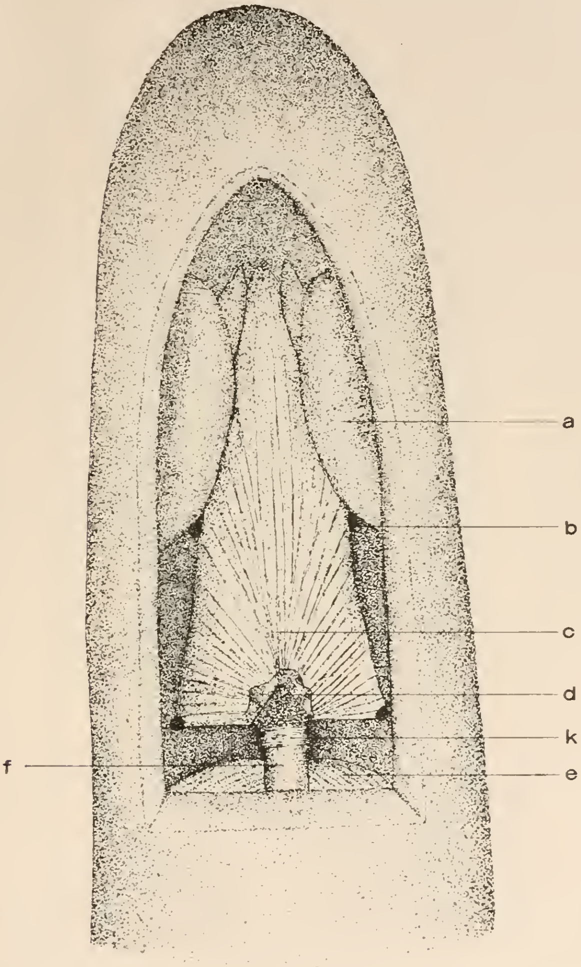

FIG. 4. Dorsal side of the head of *P. marinus* dissected to show the muscles overlying the pharynx and œsophagus. *a*, basilaris muscle; *b*, supporting cartilage; *c*, pharyngeus muscle; *d*, raphe of pharyngeus muscle; *e*, gill sac; *f*, œsophagus; *k*, posterior pharyngeus.

into the cavity in the form of a longitudinal ridge on each side.

The position of these muscles explains the difference between the size of the pharyngeus muscle viewed from the dorsal side (Fig. 4) and the actual size of the pharyngeal cavity (Figs. 1 and 5).

The outer supporting walls of the pharyngeal cavity outside of the pharyngeal muscle are: on the ventral side, the large tongue muscle; on the dorsal side, the ethmoid cartilage; on the anterior one half of the lateral walls, the basilaris muscle which expands and contracts the anterior half of pharyngeal cavity; lying dorsal to the basilaris muscles are found the large salivary glands which when the muscles act cause, according to Fürbringer (1875), a great flow of saliva.

The posterior pharyngeus is a strong bundle of muscular fibers arching over the mouth of the œsophagus between the walls of the nasal canal and that of the œsophagus (Fig. 1, *k*, and Fig. 4, *k*). This muscle lies just posterior to the pharyngeus muscle and when contracted closes off the œsophagus completely from the pharynx. When food reaches the posterior part of the pharynx, this muscle must relax to allow the food to pass on its way to the intestine.

The mucous membrane on the dorsal wall of the pharynx is continuous with that on the dorsal wall of the œsophagus, while that on the ventral wall is continuous with that on the ventral wall of the water tube. The ventral wall of the œsophagus and the dorsal wall of the water tube begin where the pharynx ends and continue caudad in close contact, parallel with the long axis of the body, to where the water tube ends, just anterior to the pericardium (Fig. 1). At the extreme anterior edge of the united walls of the pharynx and water tube there projects forward a pair of jaws which, on account of their connection with the velar valves, may be called velar jaws (Fig. 1, *j*; Fig. 5, *j*; and Fig. 6, *j*). They extend into the posterior part of the pharyngeal cavity and look like the jaws of a beetle. So close is the resemblance that at first sight one easily imagines that the animal has by some means swallowed a beetle, the jaws of which are lying in the pharynx. In Fig. 4, these jaws would lie beneath the triangular raphe (*d*).

The jaws are smooth and glossy and are covered with mucous membrane. Each is thick at the base where it unites with the other and quite rapidly tapers to its point. The space between the jaws when they are open is a broad but incomplete ellipse; when closed, the points of the jaws come together forming an entire but narrower ellipse.

The jaws extend forward from the free end of the united walls of the water tube and œsophagus and in the same plane so that the greater part of their bulk lies ventral to the œsophagus. The position is not clearly shown in Fig. 1. The plane of the jaws is moreover oblique to the long axis of the pharynx which dips

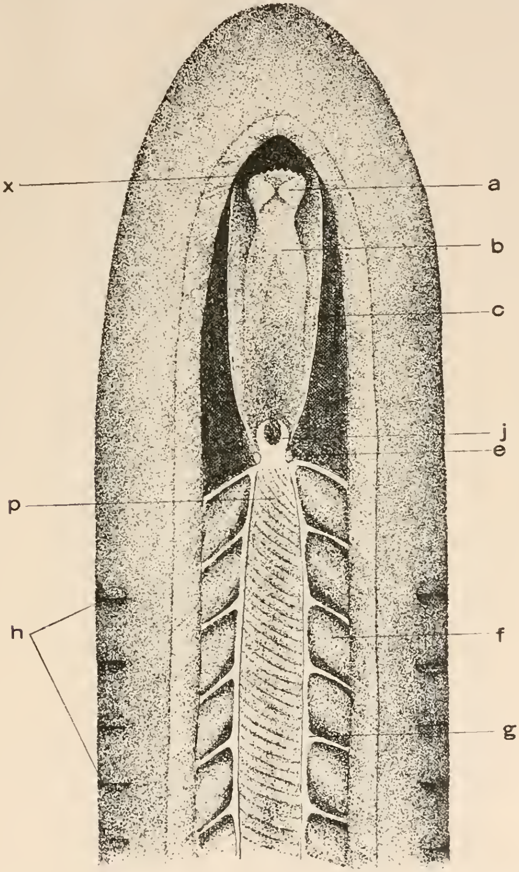

FIG. 5. Dorsal view of the head of *P. marinus* dissected so as to expose the cavities of the pharynx and œsophagus. *a*, lateral tongue lobe; *b*, tongue; *c*, pharyngeus muscle; *j*, velar jaws; *e*, posterior pharyngeus; *f*, gill sac; *g*, gill pouch; *h*, external branchipore; *p*, œsophagus; *x*, anterior lobe of the tongue.

ventrad to join the œsophagus and water tube. It results that the jaws extend obliquely across the opening from pharynx to water tube so as to intercept anything passing into the water tube. Nothing further can be seen of this apparatus from the pharyngeal cavity, but if the water tube be opened from the ventral side the velar jaws may be seen in connection with the velar valves which guard the passage between the pharynx and water tube (Fig. 6). The velar valves (Fig. 1, *l*, and Fig. 6, *d*) are two semilunar flaps which extend dorso-ventrally, one on either side

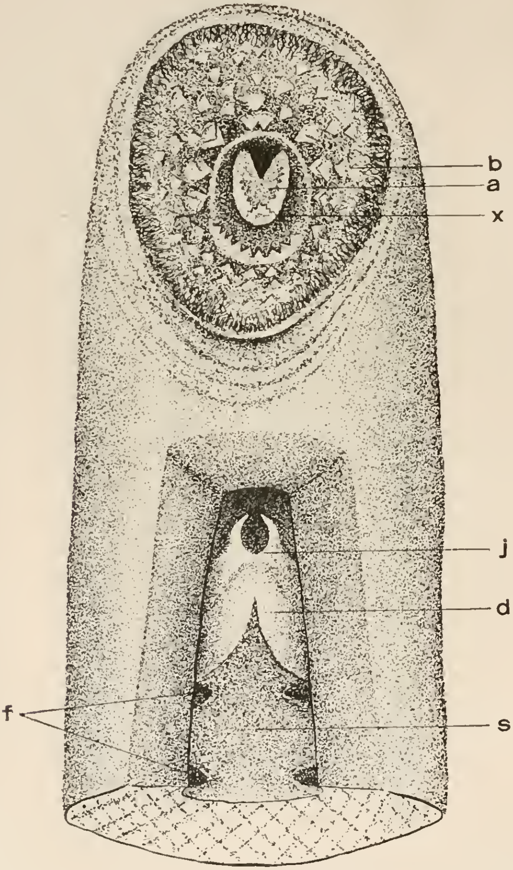

FIG. 6. Ventral view of the head of *P. marinus* with the cavity of the water tube and pharynx exposed. *a*, tongue; *b*, oral funnel; *d*, velar valves; *f*, internal branchipores; *j*, velar jaws; *s*, water tube; *x*, anterior lobe of the tongue.

of the water tube at its junction with the pharynx. They are united to one another in the middle line for 1 or 2 mm. near their dorsal ends but are free elsewhere, so that there is left between them a slit-like opening. They are concave caudally and their lateral edges are united to the water tube. They function normally to prevent water entering the pharynx from the water tube.

In order to understand the relation between the velar jaws and the velar valves the cartilaginous frame-work common to the two must be carefully dissected out. It is found to bear a striking resemblance to a pair of mechanic's nippers except that it is in one piece, whereas the latter is in two pieces. The jaws of this nipper-shaped cartilage are much smaller than the handles and have a much smaller arc between them. In fact the proportion between the jaws and handles of the cartilaginous apparatus is much the same as is found in the manufactured tool and like it tends to power in grasping. Approximation of the handles of a pair of nippers brings the jaws together. This is due to the fact that the halves of the nippers are crossed and fastened together by a pin. The two halves of the nipper-shaped cartilage are not crossed, hence when the handle is pushed inward, the corresponding jaw moves outward. The jaws of these nipper-like cartilages form the supporting skeleton of the velar jaws found in the pharynx, while the handles extend into the lateral walls of the water tube as slender cartilages which end on its ventral wall just cranial of the first internal gill opening (Fig. 6). The cartilages lie along the lateral attached edges of the velvar valves and support them.

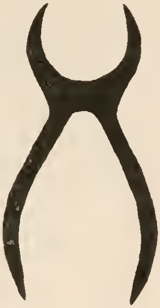

Fig. 7. Cartilaginous skeleton of the velar jaws and valves.

There are three pairs of muscles which are inserted into the nipper-shaped cartilage common to the velar jaws and velar valves. These are the velo-pharyngeus, velo-hyomandibularis internus, and the velo-hyomandibularis externus. They are sufficiently described by Fürbringer (1875), although he does not mention the velar jaws. Their combined action is to move the cartilaginous rods supporting the velar valves (the handles of the nippers) inward and outward. When the rods are moved inward, toward one another, the velar valves are relaxed and water may pass from the water tube into the pharynx; at the same time the velar jaws are opened. When the rods are separated, the valves are stretched so that they are able to close and the velar jaws are also closed.

C. *Gills.* — In order to get a clear idea of the gills it is necessary to call to mind their supporting skeleton. This consists of nine irregular vertical bars on each side. The first is placed almost immediately posterior to the styloid cartilage, the second immediately in front of the first gill cleft and the remaining seven are one just behind each of the seven gill clefts. These bars lie close to rings of cartilage, which surround the gill clefts but the bars are not continuous with the rings (Fig. 14). The vertical bars are united by four longitudinal bars; one is placed above the gill clefts and one below them, while a third lies along the side of the notochord; the fourth lies close along the mid-vental line and is connected with the corresponding bar on the opposite side. The cartilaginous pericardial capsule is connected with the ventral longitudinal cartilages at their caudal end and is very elastic. This whole basket lies external to the gill sacs. While it yields to every muscular contraction, yet it is strong enough to lend firm attachment to muscles.

Lying within this frame-work are found the gill sacs. Each gill sac is a somewhat flattened ellipsoid. It is perforated at the ends of its major axis by the external and internal branchial openings. Its shortest axis is caudo-cranial so that we may conveniently distinguish in each sac a caudal and cranial surface or wall, a lateral and medial end, and a dorsal and ventral border. These sacs are not themselves attached to the supporting cartilage, but each lies within a muscular pouch to which it is connected by muscular fibers, and this muscular pouch is in turn attached to the cartilaginous rods of the visceral skeleton on three sides, dorsal, ventral and lateral.

The openings of the gill sacs into the water tube (internal branchiopores) have slightly swollen lips. Those belonging to each pair of sacs are in the same transverse plane. The opening of each pair of gill sacs to the exterior (external branchipores) lie also in the same transverse plane, but in a plane caudal to that which passes through the internal openings of the same pair of sacs. The principal axis of each gill sac, the axis connecting the branchipores, is thus oblique to the long axis of the body and is directed from its medial end, caudo laterad (Fig. 5, *f*). The gill sac also crosses its muscular pouch obliquely. Its internal opening lies near the cephalic margin of the medial end of the pouch, while its external opening lies near the caudal margin of the lateral end of the pouch. The muscular pouches are larger than the gill sacs, so that the two lie in contact only at their openings, where they are united (Figs. 5 and 8).

When the gill sacs are examined from the outside, the lines of attachment of the gill lamellæ are seen (Fig. 8). While the walls of the gill sacs are thus transparent, they are nevertheless covered with a layer of muscle fibers (Fig. 8). These fibers are very delicate and form a thin layer which spreads over the gill sac like a spider's web and might easily escape notice. There are two of these muscles on each side of the sac. They have not hitherto been described. One muscle, *the external compressor of the gill sac* (Fig. 8, *f*), is a narrow band near the external opening of the sac. Its fibers arise from the lateral one fifth of the dorsal and ventral border of the gill sac and form over its surfaces arches with their concavities toward the external opening. The muscle functions in compressing the external one fifth of the gill sac.

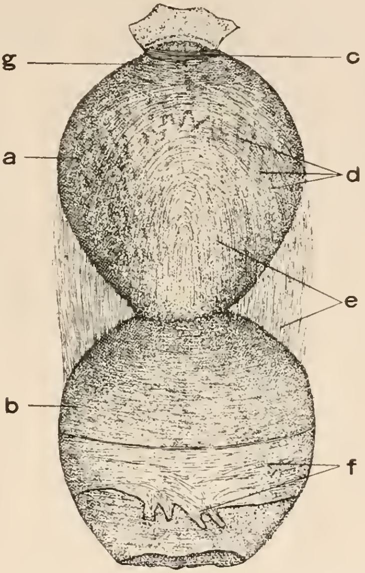

FIG. 8. Gill sac with its muscular pouch. The pouch is cut near its lateral end and reflected from the sac. *a*, gill sac; *b*, gill pouch; *c*, ental muscle; *d*, lines of the gill lamellæ showing through the gill sac; *e*, internal compressor muscle of the gill sac; *f*, deep compressor muscle of the gill pouch; *g*, external compressor of the gill sac.

The fibers of the larger muscle, the *internal compressor of the* *gill sac* (Fig. 8, *c*), arise on the medial one fifth of the dorsal and ventral borders of the muscular pouch. From this origin the fibers spread over the caudal and cranial surfaces of the gill sac not covered by the external compressor. They form arches whose concavities are directed toward the median plane. These muscles function to compress the gill sac while shortening its major axis.

The muscular pouches are placed side by side so that the cranial wall of each and the caudal wall of the next in front of it are in contact. So close do they lie that there appears to be but a single wall separating the contents of the successive pouches from one another (Fig. 5). Most writers indeed speak of the septa between the gill sacs without recognizing their double nature and the resulting muscular pouches. The double nature of these walls can be detected only by the microscope. They are very thin toward the center but become much thicker toward their borders. In preserved specimens a great abundance of coagulated lymph is found between the gill sac and its pouch. The muscular pouches are supported at their external openings by the small rings of cartilage already mentioned as lying close to the last seven vertical bars of the branchial basket (Fig. 14, *b*). These vertical and longitudinal bars of this basket lend support to the pouch on its lateral, dorsal and ventral walls. The medial wall is supported near its center by the wall of the water tube with which it is continuous and by cartilage at its extreme dorsal and ventral ends. These muscular pouches are placed in the cartilaginous basket very obliquely with the medial ends of the major axis craniad of the lateral ends. This added to the obliquity of each gill sac in its pouch causes the gill sacs to overlap each other like shingles on a roof. Nowhere can a cross section be made through the gill region without cutting two gill sacs. A line connecting the internal and external gill openings of any gill sac thus makes an angle of 45° with the long axis of the body.

The fibers of the muscular gill pouch are very difficult to follow on the outside of the pouch on account of the pigment found deposited there, but if the pouches be turned inside out, the fibers may be plainly seen crossing the flattened caudal and cranial walls (Fig. 8). These fibers form arches convex toward the external opening and end on a narrow raphe on the dorsal and ventral walls of the pouch. The action of these muscles is to compress the whole muscular pouch and to cause its lateral end to be drawn toward the water tube thus greatly shortening its long axis and that of the gill sac.

On the inner surface of the gill pouch, at about the junction of the middle and lateral thirds of its major axis, are found strong bands of muscular fibers, *the deep compressors of the gill pouches*, lying beneath the fibers of the pouch proper. They arch in the same general direction as those already described and end on the dorsal and ventral borders of the pouch as do the muscle fibers of the pouch itself. In the center of the arch, however, some of the fibers run out diagonally toward the external gill opening and are attached to the surface of the gill sac at about the junction of the fourth and lateral fifths of its longer axis. They function in compressing the gill pouch and in fastening the gill sac to the pouch. These bands are found on both sides of the pouch, but are much larger on the cranial side. This may be due to the fact that the gill sac is so placed in its pouch that a greater space is found between the pouch and the sac on the cranial side than on the caudal side. On the caudal side of the internal opening may be seen many blood vessels (branches of the afferent branchial artery entering the gill sac) and these also serve to fasten the sac to the pouch at this point.

If a gill sac be turned inside out, the gill lamellæ may be seen projecting from the inner wall and lying close together like the leaves of a book (Figs. 9 and 11). The lamellæ are found mostly upon the flattened cranial and caudal surfaces of the sac. The lateral or distal end of the sac is but slightly encroached upon by them. The medial end shows a smooth lenticular space around the internal gill opening with its long axis dorso-ventrad. From the edge of this space the lamellæ extend toward the external gill opening. Those at the middle of the caudal and cranial walls of the gill sac (*i. e.*, those lying in a direct line between the gill openings) are the longest. Thence they diminish in length dorsally and ventrally on both sides until those near the dorsal and ventral borders of the gill sac are only about 5 mm. in length. The gill lamellæ do not reach the external opening hence there is left a smooth space about the opening. This space is much wider than the one found about the internal gill opening and extends farther along the dorsal and ventral borders (Figs. 9 and 10).

The lamellæ are attached by one margin along their whole length except near the external gill opening. Here they are broadened abruptly and their ends are free. These enlarged free ends of the lamellæ are somewhat triangular and are bent dorsad or ventrad so that they overlap each other along the edge of the distal smooth area (Figs. 9 and 11). The central lamella on both the cephalic and caudal walls splits into two laminæ about 10 mm. from its free ends (Figs. 9 and 11, *b*). The laminæ of the cephalic wall end free. When those of the caudal wall reach the edge of the cartilage which surrounds the external gill opening, there extends from each a membranous sheet which forms one of the ental valves (Fig. 9, *a*; Fig. 10, *a*).

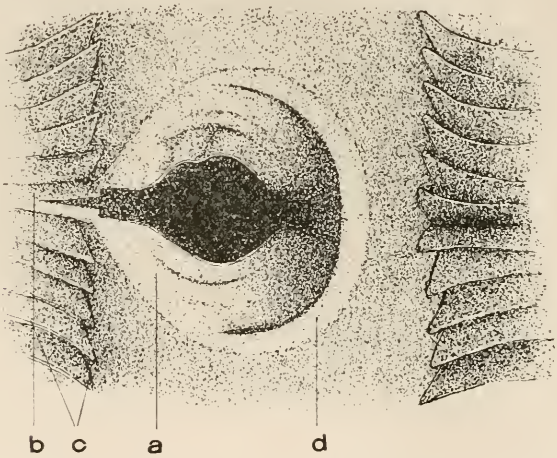

FIG. 9. A portion of the interior of the gill sac showing the external branchiopore with its ental valves in the position they assume when water is entering the gill sac. *a*, ental valve; *b*, central lamellæ which splits and forms the ental valves; *c*, laminæ ending free; *d*, cartilaginous ring of the external branchiopore.

Each ental valve is a nearly circular concave plate attached by less than one third of its circumference to the caudal half of the medial end of the external branchiopore. The branchiopore forms a short tube connecting the gill sac with the exterior. The ental valves extend laterad and at the same time dorsad and ventrad to the outer end of the tubular branchiopore to which the ectal valve is attached. Each has its concave face outward and the two cover about two thirds of the lumen of the branchiopore. Each overlaps its fellow by nearly half its dorso-ventral diameter.

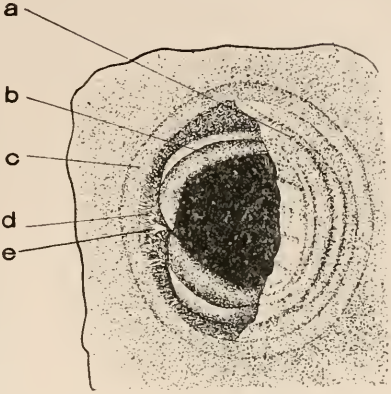

FIG. 10. The external branchiopore showing the normal position of the ectal and ental valves when water is being discharged from the gill sac. *a*, ectal valve ; *b*, ental valve ; *c*, cartilaginous ring ; *d*, buccal fringe ; *e*, point on the cartilaginous ring.

The ectal valve is a thin membrane which is attached to the cephalic half of the outer margin of the external branchiopore and stretches loosely over the cephalic half of its lumen (Fig. 9). The ental valve is attached at the internal end of the external branchiopore and on its caudal margin while

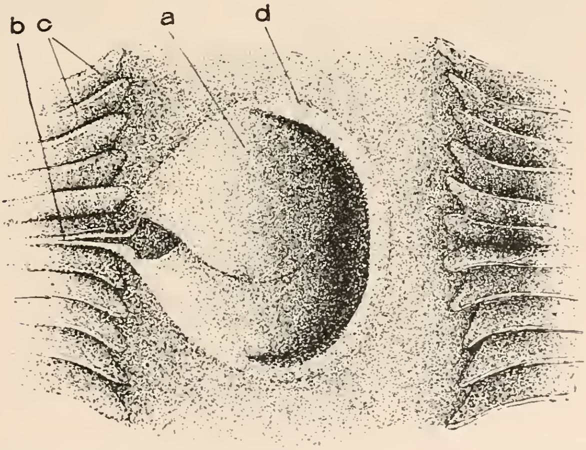

FIG. 11. Position of the ental valves when the ectal valve is stretched taut. *d*, cartilaginous ring; *a*, ental valve; *c*, laminæ of the gill sac; *b*, central divided lamellæ.

the ectal valve is attached to the external end and on the cephalic margin. When the free border of the ectal valve is tightly stretched in the cartilaginous ring (Fig. 12) the ental valves, if forced outward by pressure within the gill sac, strike against the ectal valve and close the external branchiopore thus preventing the escape of water.

If the border of the ectal valve is relaxed it does not afford a support for the ental valves which are then forced out past it by the pressure of the water and thus permit water to pass out through the external branchiopore (Fig. 10). Thus while the valves of the external branchiopore can at no time prevent the entrance of water into the gill sac, they are able under certain conditions, to prevent its exit.

The stretching of the ectal valve is effected by the action of two muscles which elongate the dorso-ventral axis of the cartilaginous ring to which the valve is attached. The first one is seen when the integument is removed and lies on the cranial side of the gill cleft. This small band of muscles lies loosely around the cephalic side of the gill opening, and is attached to the longitudinal bars immediately dorsal and ventral to the vertical axis of the opening and to the ring of cartilage (Fig. 13). This may be called the *ectal muscle*.

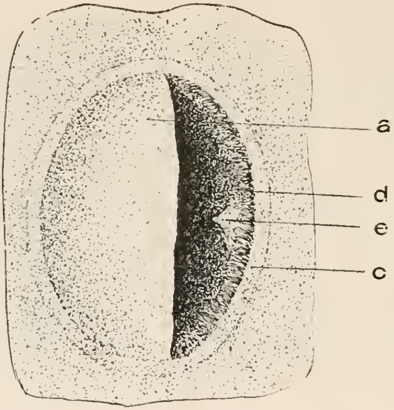

FIG. 12. The cartilaginous ring of the external branchiopore is oval in shape thus stretching the ectal valve taut. *a*, ectal valve; *d*, buccal fringe; *e*, point on the cartilaginous ring; *c*, cartilaginous ring. Figs. 11 and 12, placed back to back as here printed, form together a model of a left external branchiopore with closed valves.

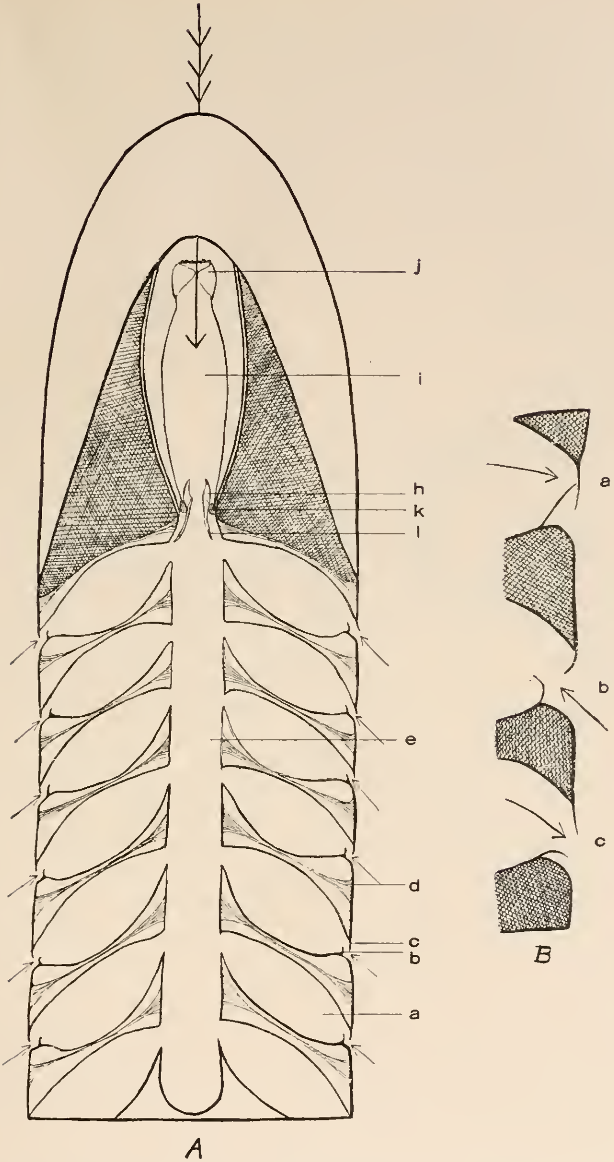

FIG. 13, *A*. A diagram of the respiratory apparatus of *P. marinus*. The possibility of the water entering the gills from the mouth and the external branchiopores at the same time is shown. *a*, gill sac ; *b*, ental valve ; *c*, ectal valve ; *d*, muscular gill-pouch ; *e*, water tube ; *i*, velar valve ; *k*, posterior pharyngeal muscle ; *h*, velar jaws ; *i*, tongue ; *j*, tongue lobe.

FIG. 13, *B*. Diagram of the ectal and ental valves of the external branchiopores of *P. marinus*. *a*, closed valves ; *b*, position of valves as water enters the gill ; *c*, position of valves when water leaves the gill.

The other half-ring shaped muscle (*ental muscle*) is smaller and extends loosely around the caudal side of the gill sac near its external opening but between the gill sac and its pouch. Thus it cannot be seen unless the muscular pouch be cut and reflected. It is attached to the same longitudinal rods of cartilage as is the ectal muscle, a little beneath the points of attachment of the latter and is attached slightly to the ring of cartilage (Fig. 8, *c*) which sends out a flat semicircular projection. This projection lies on the caudal side of the external gill opening within the ring of cartilage. It bears a slender conical rod of cartilage (Figs. 10 and 12, *e*).

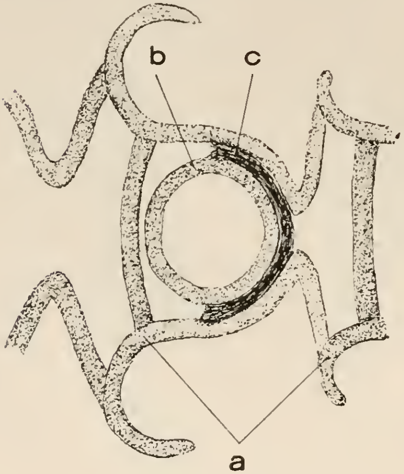

FIG. 14. The ectal muscle and its attachment. *a*, cartilaginous bars; *b*, cartilaginous ring; *c*, ectal muscle.

The ectal and ental muscles are striated and function in compressing the sides of the ring of cartilage so that the naturally circular cartilage becomes elliptical with its long axis dorso ventrad. Cuvier (1840) and Mayer (1835) speak of a circular muscle of the gill opening which lies around the ring of cartilage and serves to close the gill opening. Just how a constrictor muscle, if such a muscle were present, could so compress the cartilaginous ring as to completely close the opening is not clear.

A fringe of hair-like processes lying around the caudal border of the external gill opening deserves mention. Cephalad about 1 mm. from the middle of this fringe is the small cartilaginous projection which is the outgrowth of the cartilaginous ring before mentioned. The function of these outgrowths is not known. They may be tactile.

D. *Nasal Sac.* — The nostril is situated on the dorsal side of the head in the median line about 5 mm. anterior to the paired eyes. This nostril leads into a tube which opens directly into the nasal sac with its olfactory lamellæ. From the cranial portion of the nasal sac there continues ventro-caudad the nasal cœcum which curves around the anterior ends of the brain and notochord (Fig. 1, *f*). This nasal cœcum lies directly under the notochord and ends blindly on a line with the center of the second gill sac. The ventral wall of the tube follows the ethmoid cartilage to its posterior end and is there attached to the pharyngeus muscle as was before noted. It continues caudad from this point. At its caudal end it is parallel to the long axis of the body. The posterior part of the tube which is immediataly above the œsophagus, overlies both its dorsal and lateral walls and rests on the first and second gill sacs for 1 or 2 mm. on each side (Fig. 1).

The result of the position of the tube directly between the anterior end of the notochord and the strong muscles of the gill pouch ventral to it is that it is pressed up against the notochord at every contraction of the underlying muscles. This action of the muscles causes the water in the tube to be forced out of the nostril with considerable force. Upon the relaxation of the muscles to which the tube is attached the sac refills (Fig. 1). Thus, although the nasal tube has no direct communication with the respiratory apparatus, yet the effect is practically as though it had. At every expiration from the gills there is a corresponding expiration from the nostril and with every inspiration water passes into the nostril. The relationship existing between the expired and inspired streams of the nostril and gills has long been known, but hitherto the causes underlying this relationship have not been understood.

(*To be continued.*)

THE EYE OF CRYPTOBRANCHUS.

ALBERT M. REESE.

Since no investigations, apparently, have been made on the anatomy of the eye of the American giant salamander, *C. alleghenensis*, a brief description of that organ may be of interest.

In the living animal the eyes are quite inconspicuous, due to their small size, their lack of bright coloration, and to the wrinkled condition of the skin that surrounds them. The pupil, as seen from the surface, is very irregular in outline ; it appears as a small, jagged, black spot in the center of the gray iris. The eyes of the living *Cryptobranchus* do not differ markedly in appearance or relative size from those of *Necturus*.

In order to study their structure, the eyes, with a little of the surrounding tissue, were removed and sectioned in colloidin. Owing to the hardness of the lens it was not possible to cut very thin sections. The tissue was stained *in toto* with borax carmine, and after the sections were arranged serially on the slides they were stained with Lyon's blue. The memorandum as to fixation having been lost, it is not possible to give the method, but the results were fairly satisfactory except in the case of the layer of rods and cones of the retina. The figure represents a section through the middle region of the eye, passing through the optic nerve and the pupil.

The sclerotic coat (*Sc*) is rather indefinite in extent, and is largely chondrified. This cartilage forms a very thick capsule that surrounds considerably more than half of the globe of the eye. It is perforated, of course, at the back for the passage of the optic nerve. The walls of the cartilaginous capsule are not of homogeneous structure throughout, but are penetrated at places by ingrowths of tissue apparently derived from the choroid (*Ig*). The unchondrified portion of the sclerotic is, as has been said, of rather indefinite amount. It extends in front of the eye, between it and the superficial epithelium, to form a comparatively thick and, one would think not very transparent, cornea (*C*). In the fibrous portions of the sclerotic, as in other parts of the eye, are frequently seen large, black, many-branched pigment cells (*Ps*).

The corneal epithelium (*E*) is a direct continuation of the general epithelium of the head, there being no lids. As seen in the figure, the corneal epithelium is somewhat thinner than that of the rest of the head, and is composed of several layers of small cells with strongly-staining nuclei; the nuclei of the deepest layer are somewhat larger than the more superficial ones.

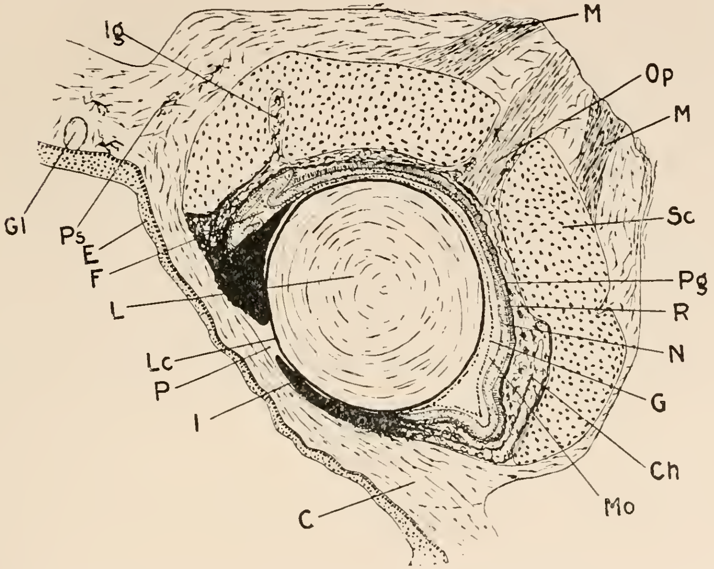

FIG. I. Section through the eye of *Cryptobranchus Alleghenicnsis*. *C*, cornea; *Ch*, choroid; *E*, epithelium (conjunctiva); *F*, fold of the retina; G, ganglion-cell layer of retina; *Gl*, gland in the skin near the eye; *I*, iris; *Ig*, ingrowth of choroid into the cartilaginous sclerotic; *L*, lens; *Lc*, lens capsule; *M*, muscles; *Mo*, molecular layer of retina; *N*, inner and outer nuclear layers of retina; *Op*, optic nerve; *P*, pupil; *Pg*, pigmented layer of retina; *Ps*, branched pigment cells; *R*, layer of rods and cones of the retina; *Sc*, cartilaginous sclerotic.

In spite of the fact that the eye seems to have practically no power of motion, there are present the usual muscles, and they are of considerable size; two of them are shown in the figure (*M*).

The choroid (*Ch*) lies in immediate contact with the inner surface of the cartilaginous sclerotic, and is continued in between the front of the lens and the back of the cornea, in the usual way, to form the iris. It is more or less closely filled with the irregular, black pigment cells that were mentioned in connection with the sclerotic. These cells are seen along the tract of the optic nerve as it passes through the cartilaginous sclerotic. No distinction of layers can be made out in the choroid, nor can a true ciliary process be determined, though a thickening and folding in the region ordinarily occupied by that structure might be interpreted as a ciliary process ; no connection of this thickening with the lens can be determined, however, and the appearance of a ciliary process seems to be largely caused by a curious folding of the retina, to be presently described. The reference line *Ch* ends in the thickening just described. In that portion of the choroid that extends in front of the lens to form the iris (*I*) the pigment cells increase to such an extent that the iris is almost or quite black, though this dark color is not so evident in the living eye.

The pupil (*P*) is, as has been said, small and irregular in outline. The retina, though not particularly well fixed in the material studied, shows at least six layers, which may be given the names usually applied to the corresponding structures in other eyes. The outermost (*Pg*) is the pigmented layer ; it is comparatively thin, but shows an inner pigmented and an outer nuclear portion. Close to the inner surface of the pigmented layer, though often torn from it, is the layer of rods and cones (*R*) ; it has been called the layer of rods and cones, though, owing to imperfect fixation, the two structures are indistinguishable. Next to the layer of rods and cones lies a deeply-staining layer (*N*) that, in favorable sections, shows an indistinct division into an outer and an inner zone, which might be called the outer and inner nuclear layers, respectively. The two nuclear layers are composed of similar round, granular elements. Inside of the inner nuclear layer is a finely-granular, nonstaining layer which probably corresponds to the inner molecular layer (*Mo*). The outer molecular layer is not distinguishable in the material at hand. The innermost layer of the retina (*G*), which seems to correspond to the ganglion-cell layer, is composed of a single row of large, rounded or oval elements that stain deeply like the elements of the two nuclear layers. No layer of nerve fibers can be seen inside of this ganglion-cell layer, nor can the connection of the optic nerve (*Op*) with the retina be determined. The ganglion-cell layer lies, as a rule, in close contact with the lens, so that there is no vitreous cavity ; the narrow space that sometimes appears between the inner layer of the retina and the capsule of the lens is probably due to a slight distortion of the eye.

One of the most striking features of this eye is the large surface covered by the retina ; it extends as is partially shown on the right side of the figure, for some distance in front of the region of the ciliary process, if the thickening of the choroid may be so called. Another striking feature of the retina is the marked fold seen in the region of the ciliary thickening. All of the retinal layers take part in this fold (*F*), which varies somewhat in complexity in different eyes, but is evidently a normal condition and not a mere artifact.

The lens (*L*) presents no striking peculiarities ; it is almost spherical in form, and completely fills the cup of the eye, so that the vitreous cavity, as has been said, is obliterated ; a small space between the front of the lens and the cornea (at the end of the reference line *P*) may, perhaps, be taken to represent the aqueous cavity. The lens is surrounded by a comparatively thick capsule (*Lc*), whose distinctness is somewhat exaggerated in the figure.

The correlation of some of the above-described structures with the habits and mode of life of the giant salamander is not difficult to determine, but in other cases the correlation is not so certain.

The flattened anterior surface of the bulb, for example, is seen in most aquatic amphibia and may be merely a measure of protection against injury by coming in contact with the rocks and other objects under which the animal may hide ; again a flattened or depressed cornea would evidently offer less friction in swimming.

A cartilaginous sclerotic is common among the amphibia, but it is difficult to see the necessity of such a heavily chondrified sclerotic in an eye that is so deeply buried as is this one. The spherical lens resembles the same structure in the teleosts, and probably indicates that the eye is especially adapted to vision at short range, the lack of transparency of the surrounding medium making long-range vision impossible, in any case. Although no definite experiments have been made along this line by the writer, it seems probable, from general observations, that *Cryptobranchus* is not very keen of sight even at short range.

The slight development of the ciliary process and the apparent absence of the ciliary muscles make it difficult to see how this animal can have any power of accommodation, since there is nothing that corresponds to the processus falciformis of the teleost eye. It is possible, therefore, that objects are clearly seen only when they are at a certain distance from the eye.

The unusually large extent of the retina may be a compensation for the slight power of motion possessed by the eye as a whole, so that the image of an object may fall upon a sensitive surface even though the object be without the ordinary line of vision.

The absence of the vitreous chamber may be correlated with the unusual refractive power of the lens, which makes a further refractive medium unnecessary, and necessitates the shortening of the eye-ball to bring the retinal surface to the focus of the highly-refractive lens.

Syracuse University,
January 28, 1905.

THE MORPHOLOGY OF THE MADREPORARIA, VI THE FOSSULA IN RUGOSE CORALS.[1]

J. E. DUERDEN.

The term fossula; as employed in the literature of rugose corals, refers to a depression or pit in the calice, due to the smaller size of the septa at that particular region. Generally only one fossula is present in a corallite (Figs. 10 and 11), but there may be three (Figs. 1 and 12), or rarely two or four. The presence of the one or more pits gives a decided bilateral character to the calice, which otherwise might be perfectly radial.

The occurrence of one or more fossulæ has always been regarded as an important characteristic of the extinct Rugosa or Tetracoralla, nothing suggestive of such being found among modern hexameral Madreporaria, and, as would be expected, various explanations have been put forward to account for their presence. The present contribution is an attempt to understand the nature of the fossulæ from the stages passed through in the development of the individual corallite.

Where only a single fossula is present it is situated towards the ventral end of the principal axis of the calice, and where three occur the two additional are lateral and symmetrically disposed; when present the fourth fossula is towards the dorsal extremity of the principal axis. The single ventral fossula is the most persistent and characteristic of the series, and may be known as the main or cardinal fossula, or better, as the ventral-directive fossula, since it is associated with the cardinal or ventral septum. The two lateral are the alar fossulæ, and are dorsal to the alar septa; the fourth is the counter or dorsal-directive fossula.

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[1] The first two parts of this series of papers appeared in the *Johns Hopkins University Circulars*, Vol. XXI., Nos. 155 and 157, and were reprinted in the *Annals and Magazine of Natural History*, Ser. 7, Vol. X., May and August, 1902. The third and fourth parts appeared in the *Annals and Magazine of Natural History*, Vol. X., November, 1902, and Vol. XI., February, 1903; the fifth part in the BIOLOGICAL BULLETIN, Vol. VII., July, 1904. The work is being carried out with the assistance of an appropriation from the Carnegie Institution.

Contributions from the Zoölogical Laboratory of the University of Michigan, No. 96.

The Alar or Lateral Fossulæ.

We may first enquire into the nature of the alar or lateral fossulæ, as represented in the calice of a rugose coral such as *Hadrophyllum pauciradiatum* (Fig. 1). The Devonian genus *Hadrophyllum* here chosen is one which Milne-Edwards and Haime,

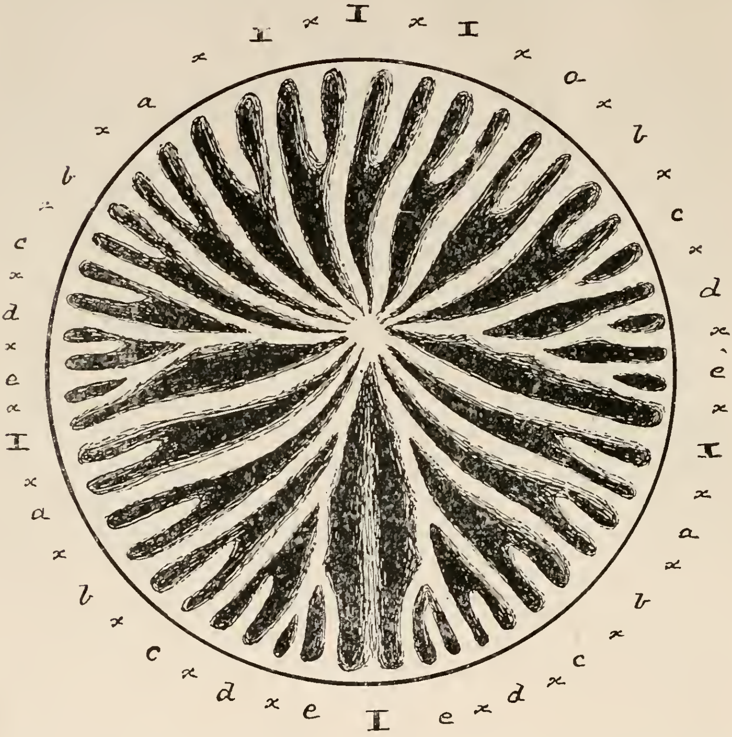

Fig. 1. Calice of *Hadrophyllum pauciradiatum*, E. & H., showing the arrangement of the septa. Here and throughout the figures the Roman numerals I indicate the six primary septa or protosepta, the letters *a–e* the secondary septa or metasepta, and *x* the exosepta making up the outer smaller cycle (see description, Fig. 7); the lower side is regarded as ventral and the upper as dorsal. The two alar fossulæ are formed by the medio-lateral group of shorter septa, *c–e*, on each side, and the cardinal fossula by the ventro-lateral group of shorter septa, *b–e*, on each side, along with the more depressed ventral directive septum (I).

The series, Figs. 2–11, shows the complete septal development of *Streptelasma rectum*, Hall. The drawings were made as the successive stages were exposed on grinding down a corallite; the middle dotted line in each septum represents the line or centers of calcification.

in their "Coralliaires" (III., p. 334), specially distinguish as having three fossulæ, one axial and two lateral, and may therefore be taken as showing the alar fossulæ in their typical condition. In the figure given each alar fossula is seen to be associated with a few smaller septa (*c–c*) which fall short of the center, the shortening varying successively in a gradual manner. Further, each of the shorter septa is inclined dorsally by its inner border towards the next larger septum and is fused with it, so that together they form a group of septa very distinct from the perfect septa, and separated from the alar septum by a somewhat deeper and wider interspace.

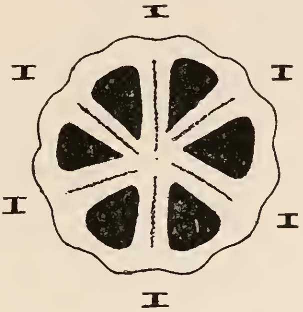

FIG. 2. Primary stage with six equal septa and six interseptal spaces or chambers. The axial septa are the dorsal and ventral directive septa, the former being the *Gegenseptum* and the latter the *Hauptseptum* of German writers; the four lateral septa are the dorso-lateral and ventro-lateral pairs, the latter being the *Seitensepta* or alar septa. The two middle interseptal chambers are the counter quadrants of palæontologists, and the two ventro-lateral are the principal or chief quadrants.

Frequently, however, between the primary alar septum and the group of incomplete septa on its dorsal aspect there is scarcely any special depression or pit present, such as is implied in the term fossula; even individuals of the same species vary much in this respect. Hence it will give a more precise morphological significance to the term if it be extended so far as to refer to the smaller, grouped condition of the septa, whether or not they are separated by a special depression from the alar septum. The subsequent discussion of the alar fossulæ will therefore have reference more to the group of smaller septa in this region than to any actual depression in the calice with which they may or may not be associated. Where the grouping occurs it is always a conspicuous feature of the calice.

The significance of the shorter septa associated with the alar fossula in *Hadrophyllum* can be understood by comparing the series of figures (Figs. 2–11) representing the complete septal development of *Streptelasma rectum*, as revealed by successive sections of a single corallite.[1] It is seen that each alar or lateral region is that at which new septa (*a–e*) are successively added to the primary six. Furthermore, the additions take place in such a manner that the newer, shorter septa are for some time inclined towards the older, and are fused with them in a unipinnate manner by their inner borders; it is only towards the close of development (Figs 10, 11) that all the septa become free, and are then radially arranged. This inclination and fusion of the newer with the older septa, as well as their smaller size, gives a distinctive character to the alar regions in *Streptelasma* during the various developmental stages, and usually results in a special interval or interseptal space on the dorsal side of each alar septum.

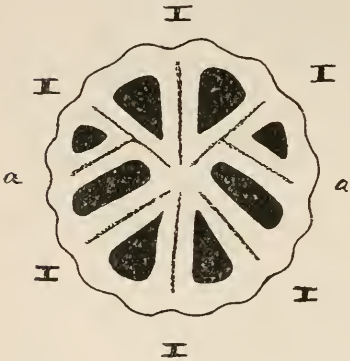

Fig. 3. A bilateral pair of metasepta (*a*) has appeared, a member within each of the middle chambers or counter quadrants, inclined towards its corresponding dorso-lateral primary septum.

If now the alar regions of Figs. 2–10 be compared with those of Fig. 1, the two alar fossulæ in *Hadrophyllum* are seen to correspond with the two lateral regions of addition of new septa in *Streptelasma*, and both are within the two middle of the six primary interseptal chambers. Hence each alar fossula in the adult corallite of *Hadrophyllum* really corresponds with ontogenetic stages in the establishment of the septa in *Streptelasma*, the septa concerned being those immediately dorsal to the primary alar septum.

That no alar fossula is present at the more mature stages of *Streptelasma* (Figs. 9–11) is due to the fact that as the coral attains its full development the septa become free from one another at their inner border, and at the same time become equal in size and situated at equal distances apart, thereby presenting a more truly radial disposition. On the other hand in the mature corallite of *Hadrophyllum* the septa of the alar region do not reach the radiate condition; they retain their unipinnate arrangement throughout, and, as a consequence, the alar regions remain sharply separated from the rest of the corallite both by a special arrangement of the septa and by an interval.

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[1] The septal sequence here illustrated is in accordance with Kunth's well known law of septal development found to be characteristic of the Rugosa. Hitherto, it has been generally assumed that only four primary septa are present in tetracorallids whereas six actually occur. However Haeckel's term Tetracoralla has still an appropriateness since the subsequent septa are formed within only four of the six primary interseptal chambers in contrast with the six in Hexacoralla.

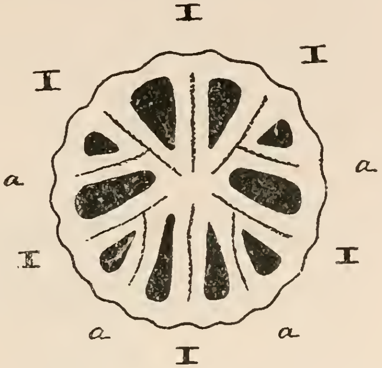

FIG. 4. A stage a little beyond that in Fig. 3. A corresponding pair of septa (*a*) has appeared within the two ventro-lateral chambers or principal quadrants, each member inclined towards a ventro-lateral (alar) primary septum.

The series of sections of *Streptelasma* further proves that the alar fossula is situated on the dorsal side of the alar septum; the latter takes no direct part in forming the depression, but merely constitutes its ventral boundary. As shown in Fig. 2, the right and left alar septa are the ventro-lateral pair of the six primary septa, and all the additions in the middle interseptal chambers necessarily take place dorsal to them. In sections the new septa are inclined by their inner border towards the dorso-lateral pair of primary septa, and are thus more nearly parallel with the ventro-lateral (alar) septa.[1] Sometimes, as in *Anisophyllum agas-*

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[1] In giving the septal plan of a tetracorallid as viewed from above Delage & Hérouard ("Traité de Zoölogie Concrète," II^{2}, p. 692, Fig. 973) project the newer septa of the principal quadrants upon the cardinal septum and parallel with the alar septa, while those in each counter quadrant are projected upon the alar septum and are parallel with the dorsal directive or antipodal septum. The figure of *Hadrophyllum* here given, and also those showing the septal development of *Streptelasma rectum*, prove that this is not the true relationship of the inner ends of the metasepta within the calice. In each quadrant the inner end of each septum is from its origin directed towards the next older member of its own series. I have met with no instance in which the later developed septa are inturned towards and fused with the alar and axial directive septa, as seems to be usually assumed from the appearances presented by the external ridges and grooves alone. The explanation which Bourne gives (Lankester's "Treatise on Zoölogy," Art. Anthozoa, p. 73, Fig. 35) of the schematic representation of the septa of a zaphrentoid coral is manifestly self-contradictory as regards the sequence.

sizi, E. & H., the alar septa are much larger than any subsequently developed, and may thus emphasize the fossula adjacent to them. On the other hand, it is very doubtful if the alar septa themselves are ever smaller than the other principal septa, or are situated within the fossular depression, as is sometimes assumed in palæontological works.

Alar fossulæ of a like nature with those in *Hadrophyllum pauciradiatum* have been studied in other tetracorallids, especially in *Microcyclus discus*, Meek & Worthen, *Anisophyllum Agassizi*, E. & H., and also in *Hadrophyllum glans* (White). In these there is the same grouping of successively smaller septa, giving to the alar region its distinctive character. A still larger number of zaphrentoid corals, however, are found to resemble *Streptelasma rectum*:[1] during the earlier stages of development their new septa are related one to another just as they are in the adult of *Hadrophyllum*, while on approaching maturity they become equal and more strictly radial; they all, in fact, pass through a *Hadrophyllum* stage.

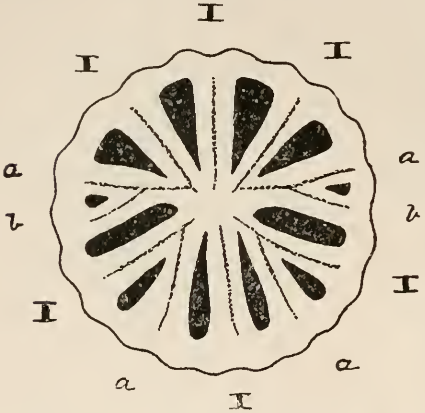

FIG. 5. A second pair of septa (*b*) is now present, one in each of the middle chambers, inclined towards the metasepta (*a*).

The true morphological significance of the difference between adult forms like *Hadrophyllum* with alar fossulæ and others like *S. rectum* in which they are wanting is thus only ontogenetic. In the one development does not proceed sufficiently far as to establish approximate radial symmetry, and the adult corallite retians the bilaterality of development of its septa; while in the other the bilateral symmetry of growth becomes replaced by a radial disposition of the parts. Whenever alar fossulæ are present they represent an incompletion in the establishment of the newer septa of the alar region, as compared with species in which no alar fossulæ are represented; they have only a developmental significance, and would not correspond with any structural peculiarity of the fully developed polyp. As would naturally be expected from such an explanation even individuals of the same species may vary much with regard to the presence or absence of alar fossulæ. In the calice of some specimens of *Microcyclus discus*, for example, there is a distinct grouping of the alar septa, while in others the septa have become perfectly radial, and no alar region is distinguishable.

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[1] It may be well to state that in the course of the investigations the septal development has been followed by means of serial sections in various species of the following genera comprised within the families Cyathaxonidæ, Palæocyclidæ, and Zaphrentidæ, namely, *Cyathaxonia*, *Duncanella*, *Palæocyclus*, *Hadrophyllum*, *Microcyclus*, *Streptelasma*, *Zaphrentis*, *Lophophyllum*, and *Anisophyllum*. Representatives of the families Cyathophyllidæ and Cystiphyllidæ, particularly the colonial forms, are found to be unsuitable for such studies.

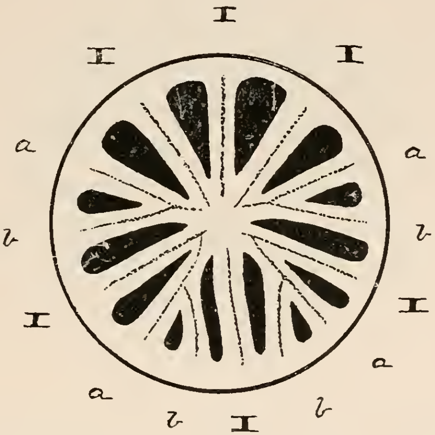

FIG. 6. A corresponding second pair of septa (*b*) has appeared within the ventro-lateral chambers.

The alar fossulæ represent regions where new septa are being added, and the interspaces may reasonably be expected to be different from the other septal interspaces, and likewise to vary at different stages according as the new septa are just appearing or are nearly fully developed.

From developmental studies the conclusion is thus reached that in all ordinary species of zaphrentoid corals the septa of the alar region pass through a fossular stage as here understood, whether or not the adult arrangement be radiate; or, stated in another way, the presence of alar fossulæ in an adult corallite is a retention of a developmental characteristic.

The Cardinal or Ventral Fossula.

Enquiry may now be directed into the nature of the cardinal or ventral-directive fossula. This is the most important of the series, being usually represented in mature calices even when all traces of the alar fossulæ have disappeared. Further, its constitution is by no means so simple as that of the alar fossulæ.

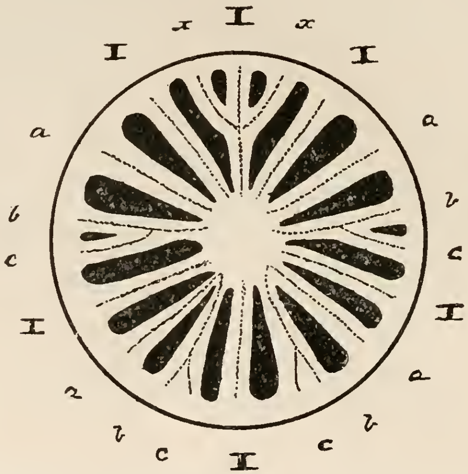

FIG. 7. A third additional metaseptal pair (*c*) has appeared within both the middle and ventral chambers, the middle pair developing first. A pair of septa (*x*) has also appeared, one within each of the dorso-lateral primary chambers, fused with the dorsal directive. In relation to the polyp the septa which appear midway between the principal septa are exosepta, and always constitute the outer, smaller cycle in the mature calice; the larger septa, constituting the inner larger cycle, include both the protosepta and metasepta, and are entosepta.

As represented in *Hadrophyllum* (Fig. 1) the cardinal fossula is associated with two groups of successively shorter septa (*b–e*), a group being situated on each side of the cardinal or ventral directive septum, and, in addition, the cardinal septum (*I*) is itself smaller than the other fully developed principal septa. The members of each ventro-lateral group of smaller septa (*b–e*) are related to one another in the same manner as are the members in an alar fossular group (*c–c*), that is, the shorter septa are turned towards the successively larger, and are united with them by their inner ends in a unipinnate fashion. In general also the space on each side between the group of newly added septa and the axial septum is greater than the other septal interspaces and more pit-like.

Again comparing the adult calice of *Hadrophyllum* with the developmental series of sections of *Streptelasma* in Figs. 2–11,

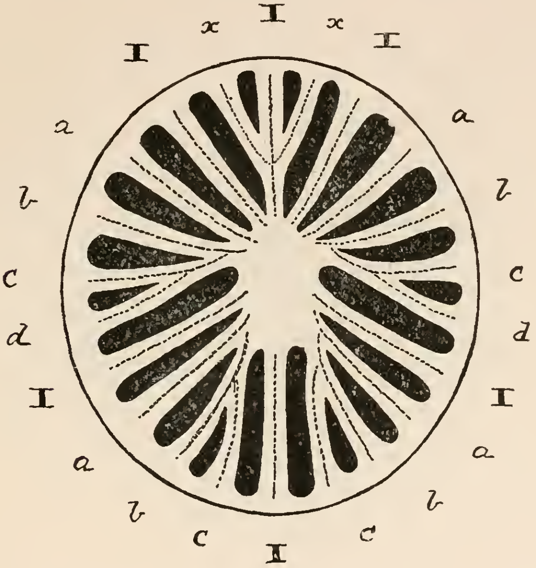

FIG. 8. A stage somewhat in advance of that in Fig. 7. An additional septum (*d*) is present in each middle chamber, while the two pairs, *c*, are much larger. The section shows very clearly the inclination of the septa towards each other in the alar and ventro-lateral regions, only the oldest septa reaching the columella (cf. Figs. 1 and 12).

it is seen that the fossular region on each side of the cardinal septum is a region where the addition of new septa takes place, just as is that on the dorsal aspect of each alar septum. Likewise, the new ventral septa in *Streptelasma* are inclined towards the older and fused with them until the mature condition is reached, when they become free, of the same size as the others, and more nearly radially arranged (Figs. 10, 11). Therefore the cardinal or ventral groups of shortened fused septa in *Hadro-*

phyllum represent a developmental stage when compared with the mature condition of such a form as *Streptelasma ;* as in the case of the alar fossulæ, a developmental stage in *Streptelasma* persists as the adult condition in *Hadrophyllum.* The axial cardinal fossula of *Hadrophyllum* is thus shown to be associated with two groups of incompletely developed septa, a group on each side of the cardinal directive septum, while at the same time the latter is much smaller than the other primary septa. Such a fossula as a whole may be spoken of as compound, while those in the two alar regions are simple fossulæ.

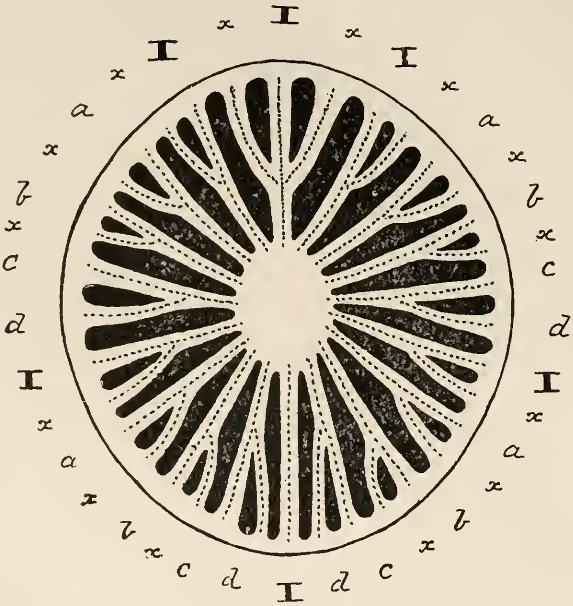

Fig. 9. Exosepta (x) have now appeared in association with most of the fully developed septa, namely, all the protosepta (except the ventral directive) and the metaseptal pairs a and b. The exosepta are at first turned towards and fused with their corresponding dorsal entoseptum.

Studies on other species of rugose corals show that throughout the group the cardinal fossula in the adult calice is constituted either on the *Hadrophyllum* or *Streptelasma* plan ; rarely, as in *Anisophyllum*, the cardinal directive septum is much larger than the other primary septa and then the fossula is really double, a depression on each side of the large axial septum corresponding with the smaller, newly added septa.

As shown in the various figures the cardinal or ventral-directive septum is smaller than the other principal septa, and remains thus when all the other septa have become equal; the axial fossula is then represented in the fully developed calice by the smaller cardinal septum alone, without a special lateral depression on each side. Such a condition clearly calls for a different explanation from that given for the alar fossulæ, or for the lateral parts of the cardinal fossula; these have been found to correspond with ontogenetic stages of species which attain nearly perfect radiality in the adult, while the ventral-directive septum in most instances remains shorter even within the mature calice. Manifestly the small directive septum must correspond with some

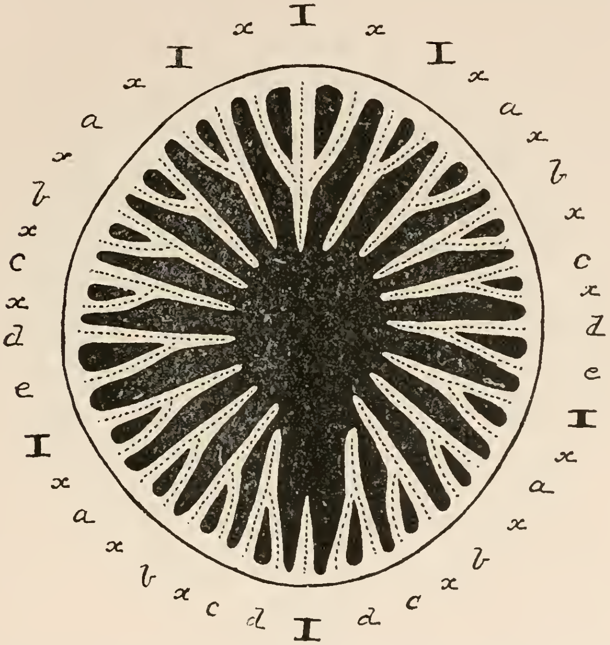

FIG. 10. Section towards the upper part of the corallite. The septa are now free from the columella, and the ventral directive septum is smaller than the others. More exosepta and metasepta have appeared than in Fig. 9, each exoseptum being still fused with its corresponding entoseptum.

peculiarity in the polyp itself, an axial peculiarity situated towards the ventral side.

In a paper already published (*Ann. & Mag. Nat. Hist.*, May, 1902), I have given good reasons for supposing that of all modern Anthozoa the living Zoantheæ are most nearly related to the extinct Rugosa. In zoanthids the mesenteries beyond the primary six are added in such a manner as would give the septal sequence characteristic of the Rugosa, that is, the sequence represented in Figs. 2–10; the only difference is that in modern zoanthids the new mesenteries are added within only the two ventral of the six primary chambers, whereas in the rugose polyps they were added within four of the six primary chambers — the ventral and middle pairs. This difference is, however, but one of detail compared with the actual order of development

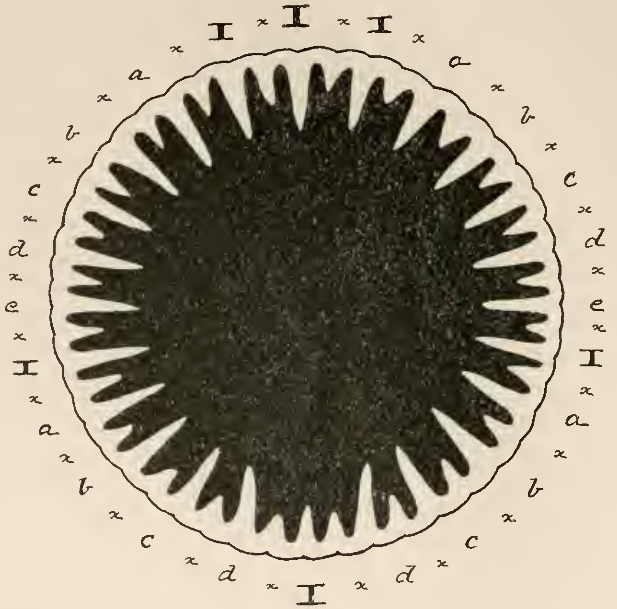
I x I x I
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FIG. 11. A section near the upper limit of the corallite. All the septa are now more strictly radial, and those of the inner and outer cycles regularly alternate. There is no distinction in size between the six protosepta (I) and the various metasepta (*a–e*), except as regards the ventral directive septum, the smallness of which gives rise to the simple axial fossula.

of the mesenteries and septa. It is the manner of appearance of the septa beyond the primary six which separates the Rugosa from modern hexameral corals, a separation of the same significance as that by which zoölogists distinguish cyclic hexameral actinians from zoanthid polyps.

The presence of the smaller axial septum in the cardinal fossula of the Rugosa serves to confirm in a peculiarly direct manner the relationship of the group to the living Zoantheæ. As is well known, one of the characteristics of the zoanthid polyp is the occurrence of a single gonidial groove, sulcus, or siphono-

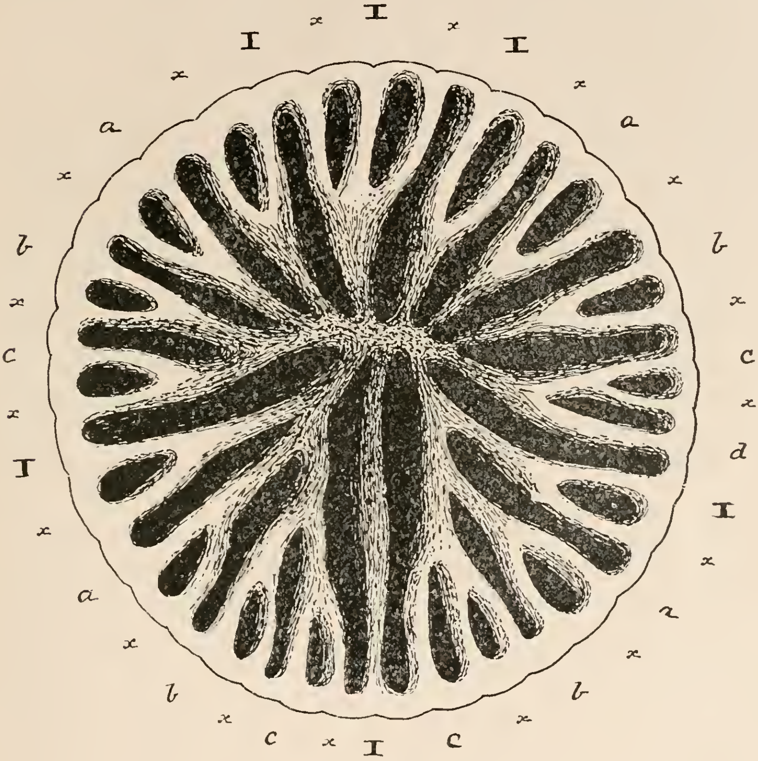

FIG. 12. Arrangement of the septa in a young corallite of *Streptelasma profundum* (Owen). At this early stage the septa are arranged in a manner very similar to those in the mature corallite of *Hadrophyllum* (cf. Fig. 1), while in the fully developed corallite they are nearly radial. The septa show a slight inequality in number on each side, a condition also usually met with in recent zoanthid polyps.

glyph along the ventral extremity of the stomodæum, as compared with the two opposite grooves (dorsal and ventral) usually present in hexactinian polyps. The single groove in zoanthids is usually well-developed, and its walls often project for some little distance within the ventral directive entocœlic chamber, and are frequently continued into the polypal cavity beyond the rest of the stomodæal wall. It is strongly ciliated and is concerned chiefly in the circulation phenomena of the polyp, sometimes remaining open when the mouth is otherwise closed by the approximation of the lips.

The occurrence of the single gonidial groove gives a marked contrast to the two extremities of the stomodæum of zoanthid polyps, and a decided bilateral character to transverse sections. Manifestly it is the presence of just such a structure which we should expect to result in a diminution of the axial septum were a skeleton formed beneath it; indeed, with such a stomodæum, having a groove at one end and not at the other, we could scarcely expect that the two extremities of a corallite would be alike. In the absence of the rugose polyp itself, no surer proof of the relationship of the group to the zoanthids could to my mind be adduced than that which admits of the correlation of the simple cardinal fossula with a ventral stomodæal groove.[1]

On the above explanation it may reasonably be asked why no corresponding axial fossulæ are found in the calices of recent corals. In answer to this it can be affirmed that as yet we have no certain instance of the presence of a gonidial groove in the stomodæum of modern coral polyps. In the polyps of over thirty species of West Indian corals which I have examined there was no evidence of such a groove, and the same can also be said of a like number of Pacific corals. The stomodæum of coral polyps is comparatively short, and presents the same histological structure all round, showing there is very little if any physiological differentiation associated with its extremities. In this respect living coral polyps differ from the closely allied skeletonless hexactinians, where a gonidial groove usually occurs at each extremity of the stomodæum.

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[1] Other evidences of bilaterality and axial differentiation are sometimes presented by zoanthid polyps. In living examples of *Zoanthus* the axial tentacle over the ventral directive entocœle is frequently larger than any of the others; sometimes the coloration along the dorso-ventral axis is different from that elsewhere; and I have also found the disc to be actually grooved or depressed along the axis. The Ceriantheæ are also characterized by the possession of a single siphonoglyph, but the manner of addition of the mesenteries in this group precludes any relationship with the Rugosa.

An examination of other species of rugose corals demonstrates that the cardinal fossula is somewhat similarly constituted throughout the group. The two extremes of its development are the complex condition met with in forms like *Hadrophyllum* and the simple modification present in the mature calice of *Streptelasma.* In the one we have it associated with a grouping of incompletely developed septa on each side of a feebly developed axial septum, while in the other it is represented by the smaller axial septum alone. The fossula in the first has an ontogenetic as well as a morphological significance, while in the second it is correlated with a structural peculiarity of the mature polyp. In zaphrentoid corallites in which the calcareous material has been replaced by silica, and the septa are exposed throughout their vertical length,[1] the entire series of developmental changes undergone by both the alar and the cardinal fossulæ can sometimes be observed at a glance, confirming the results obtained from a series of sections like that shown in Figs. 2–11.

The condition in rugose corals with the newly added septa arranged bilaterally in four groups, distinct from the other septa, I propose to term the *Hadrophyllum-stage*, since it is so characteristically displayed in this genus. In general it will be associated with three fossulæ. In its significance the stage is comparable with that of the *Edwardsia* or *Halcampa*-stage met with in some mature actinians and the polyps of modern corals, and passed through in the ontogeny of others. All the simple Rugosa, namely those embraced within the group Zaphrentoidea, exhibit the *Hadrophyllum*-stage for a longer or shorter period during their development; in some it is retained at maturity, while in others it is replaced by a more nearly radial condition of the septa.

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[1] For beautifully perfect silicified specimens of *Streptelasma profundum* (Owen), with all the interseptal matter removed by decalcification, I am indebted to Prof. F. W. Sardison, and for others from the geological collections of the University of Michigan to Prof. I. C. Russell. For many other species of Rugosa from the National Museum I am under obligations to Prof. C. Schuchert, and from the British Museum to Dr. F. A. Bather.

The ontogeny of the various forms shows that the shorter septa associated with the fossulæ cannot be considered as representing a cycle or cycles different from the others. Polycycleism, as we know it in the hexacorallids, does not occur in the tetracorallids; however many septa may be developed there are only two cycles represented, the inner larger septa being entosepta while the outer shorter are exosepta. The entosepta include the six primary protosepta and the later metasepta, some members of the former being at times larger than the rest (*Anisophyllum*). In *Menophyllum* the metasepta formed within the middle and ventral chambers differ greatly in size, those in the two ventral chambers being the larger. In this genus three fossulæ are said to occur, the two lateral situated as before on the dorsal aspect of the alar septa; the alar septa are not, as is generally assumed, included within the fossula itself.

As stated in the introduction, a fourth or dorsal-directive fossula is sometimes present in rugose corals, but this occurs very rarely, *Omphyma* being given as an example. Among a collection of *Zaphrentis compressa*, E. & H., from Spergen Hill, Indiana, I have found several in which there is in the mature corallite a conspicuous fossula towards both the dorsal and ventral extremities of the principal axis, the ventral fossula being the more pronounced. In the majority of specimens, however, there is no hint of the dorsal pit. The dorsal fossula would appear to have no developmental significance, since the septal arrangement is there the same as in other species of this genus; rather, it would seem to correspond with some peculiarity of the adult stomodæum, perhaps with the longer continuation of its dorsal extremity.

Radial and Bilateral Symmetry.

A comparison of rugose corals having bilateral symmetry with those in which all the septa are radially arranged introduces considerations with regard to symmetry in corals generally. Mature corals and actinians, like most cœlenterates, are characterized by a more or less perfectly radial symmetry as regards their mesenteries, tentacles, and septa, yet throughout the course of their development these organs follow a decided bilateral method, both in the earlier and later stages.

So far as concerns modern corals I have lately shown (Biol. Bull., July, 1904) that the septa, correlatively with the mesenteries, present very marked bilateral phases during nearly the whole of their ontogeny; it is only as the organs attain maturity that they become radially symmetrical.

Likewise the study of the development of a large number of rugose corals by the method of grinding proves that they are all characterized by a bilaterality of growth after the protoseptal stage; in the majority of species the bilaterality passes into a radial stage, but in some it is retained at maturity. The bilaterality of the Rugosa is however of an altogether different nature from that of modern Madreporaria; the septa in rugose corals are added as bilateral pairs at only four regions of growth, two on either side, whereas in modern corals the additions are made all round the periphery within the six primary interseptal spaces. Notwithstanding these developmental differences a more or less radial symmetry is reached by the adults of both groups. Little or no importance is thus to be attached to the old distinction that the Rugosa are bilaterally symmetrical while recent corals are radial; the species of both are ontogenetically bilateral, and the degree of radiality attained by the adult varies much in forms otherwise closely related, or even in individuals of the same species. Developmental bilaterality and mature radiality are just as much a feature of the extinct Tetracoralla as they are of recent Hexacoralla.

The more or less perfect radiality of the different groups of the cœlenterates as a whole is reached from very different developmental conditions, as can easily be seen from a survey of what is known with regard to the early stages of the organs in the scyphomedusæ, actinians, zoanthids, cerianthids, and recent and fossil corals. It follows that the adult radiality of organs which arise in such widely divergent manners in no way implies morphological relationship among the animals possessing them. Rather, it is an adaptation to the uniformity of environmental influences on all sides to which sessile or floating organisms are subject. The manner in which the organs arise seems immaterial; however varied the origin the same end is attained at maturity. The feature in the Rugosa here sought to emphasize is that while the majority pass through their bilateral developmental stages and attain practical radiality, some retain the early bilaterality, evidenced by the presence of one or more fossulæ, The single stomodæal groove of the polyp, correlated with an axial fossula in the skeleton, is the only deep-seated character uninfluenced by the equality of the environmental influences, and usually precludes the attainment of perfect radiality among the Rugosa.

Why should certain rugose corals when mature retain the developmental bilaterality of the *Hadrophyllum*-stage while others continue their growth until their septa attain the radial condition? The former has been shown to be an early ontogenetic stage of all forms, hence rugose corals with alar fossulæ and a compound cardinal fossula are at a lower developmental stage than those attaining radiality. How far the differences are chronological seems uncertain, for some of the earlier Rugosa are as nearly radial as their later representatives, and the adult *Hadrophyllum*-stage was apparently no more common in earlier than in later Palæozoic times. According to Zittel ("Text-book of Palæontology," *Eng. Ed.*, p. 74), the distribution of certain genera characterized by a well-marked *Hadrophyllum*-stage is as follows: *Baryphyllum*, Devonian; *Hadrophyllum*, Devonian; *Anisophyllum*, Ordovician to Devonian; *Menophyllum*, Carboniferous Limestone; *Microcyclus*, Devonian. Others having a more nearly radial disposition of the septa in the adult are distributed in time as follows: *Streptelasma*, Ordovician to Carboniferous; *Zaphrentis*, Silurian to Carboniferous; *Cyathaxonia*, Carboniferous Limestone; *Duncanella*, Silurian; *Palæocyclus*, Silurian. Obviously both forms of corallite, bilateral and radial, were living together during the greater part of the Palæozoic age.

When the majority of trifossulate zaphrentoids are compared with the radial forms, the former are seen to be short, flat, or trochoid corallites, the latter long and conical; also the trifossulate species are usually smaller and have a fewer number of septa. It would seem that corallites with considerable vertical height have had the opportunity, as it were, to outgrow their developmental bilaterality, and their septa have assumed the radiality of maturity, while this has not been the case with the short flat forms; wherever continuous vertical growth takes place, without the addition of new septa, radiality tends to be assumed by the septa already formed. In elongated forms a considerable vertical interval occurs between the insertion of each new bilateral pair of septa and an approximate radiality is attained, to be again destroyed on the addition of another pair. Somewhat similar changes have been shown to be also characteristic of the growth of modern hexameral corals; the developmental stages are bilateral, but between one stage and another an approximate radiality is reached. As regards the Rugosa it may be accepted as a general rule that developmental bilaterality at maturity is associated with shortness of calicular form.

It does not appear from systematic works that the presence or absence of fossulæ has proved to be a character of much taxonomic value. It is conceivable that forms otherwise closely allied may vary as to whether or not the septa at maturity attain the radial arrangement. The facts already adduced prove, however, that in any series of closely allied forms those with well marked fossulæ represent an earlier phylogenetic stage than those attaining radiality, but beyond this it does not yet seem possible to go.

Explanation of the Fossula.

Concerning the significance of the fossulæ the view here maintained is that they are due to two distinct structural features: (1) a grouping of smaller incompletely developed septa at the region of growth within the middle and ventro-lateral pairs of the six primary interseptal chambers (counter and principal quadrants); (2) in the case of the cardinal fossula only, a smaller ventral directive septum correlated with the presence of a single stomodæal groove in the polyp. The alar fossulæ are altogether the result of the first condition, while the cardinal fossula is either dependent upon a combination of both the first and second causes, or is entirely due to the second. Exceptionally, as in *Anisophyllum*, the cardinal fossula is due only to the first, when it appears as a double depression.

Other explanations of the origin of the rugose fossulæ have been offered. The view hitherto generally accepted is that which regards them as a sort of chamber for the hypertrophied mesenteries bearing the sexual products of the polyp. This was first suggested by Moseley (*Q. J. M. S.*, XXII., p. 394), from his studies of the living coral *Seriatopora*, in the following words: "The presence of the deep pits in *Seriatopora* for the reception of the single pair of generative mesenteries and their hypertrophied mesenteries may possibly explain the pits occurring amongst the septa of some palæozoic corals which may have had a similar function."

This theory has met with general acceptance only among English writers, particularly Nicholson, Ogilvie, and Bernard. It is founded entirely upon conditions met with in the modern *Seriatopora*. In the polyps of this genus, only the six primary pairs of mesenteries are present, the first developmental pair being by far the largest and bearing the gonads. In other corals, *e.g.*, *Pocillopora*, *Porites*, and *Acropora* (*Madrepora*), a like inequality, though to a less degree, is met with wherever only the six primary mesenteries occur, but as further cycles of mesenteries appear the six primary pairs become equal and are all fertile; moreover, in zoantharian larvæ the first pair of mesenteries often greatly exceed the others in the extent of their development. As regards their mesenteries the adult polyps of *Seriatopora* are merely at an early phylogenetic stage, and the skeletal pits are correlated with this. Our knowledge of the Anthozoa as a whole gives no support whatever for thinking that any of the mature rugose polyps had only two or even only a few reproductive mesenteries. Rather, the large number of principal or first cycle septa occurring in rugose corals, all more or less equal, gives good reason for assuming that a correspondingly large number of mesenteries would be fertile, as in the polyps of recent Zoantheæ, and also that such fertile mesenteries would be distributed all round the polyp. Moreover, Moseley's suggestion would explain only the occurrence of the pair of deep pits, one on each side of the primary septum; it would not account for the smallness of the axial septum itself.

Bernard[1] (1904, p. 10), while accepting that the use assigned the fossula, as a sort of crypt for the sexual products, is probable enough, considers that this need not have been the cause of its origin. Rather, he would explain its presence on the mechanical assumption that at an early stage in its development the coral falls over, and in recovering the upright position the soft polypal parts detach themselves from the base of the prototheca in such a way as to bag down, and thus produce a pit or depression on the floor of the calice. Bernard thus expresses it: "The fossula has a very simple explanation, if the assumption of the falling over is correct. As the soft parts detach themselves from the base of the prototheca they might be expected to bag down, and they will continue to be acted upon by gravitation and drawn over towards the convex side of the coral until the vertical position has been regained. It is possible that this bearing over to the side may be due to the efforts of the polyp itself to bend up, but gravitation in a *causa efficiens*."

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[1] Bernard, H. M., "The Prototheca of the Madreporaria, with Special Reference to the Genera Calostylis, Linds., and Moseleya, Quelch," *Ann. Mag. Nat. Hist.*, Ser. 7, Vol. XIII., Jan., 1904.

Such a purely mechanical explanation is very unsatisfactory when we consider how hypothetical is the conception of any general falling over of the prototheca, and also of the influence which this would have upon the polyp itself as well as upon the corallite; it is difficult to conceive of the bagging down of the polypal tissues always at a definite region of the calice on each side of the cardinal septum. The view which I have submitted above, that the simple cardinal fossula is correlated with the presence in the living polyp of a gonidial groove or siphonoglyph, is more in harmony with the facts of anthozoan morphology, while there can be no question as to the significance of the grouping.

Bernard fails to see the evidence for the existence of more than one true fossula in any coral examined by him. He finds this usually on the convex or "dorsal" side, this being the side which I have here termed ventral, as being more in agreement with the accepted anthozoan terminology. The occasional presence of the fossula on the concave side he attempts to explain as dependent upon a shallower more open prototheca than that in which it forms on the convex side.

Some of Bernard's other statements seem so contrary to all that we know of the nature and development of the Rugosa that

I refer to them here, although not directly bearing upon the fossula. He makes the supposition (p. 11) that "The falling over of the prototheca will explain the departure from a strictly radial symmetry of the septa seen in these curved Palaeozoic corals." He also remarks: "Further, it has long been known that, as such corals gradually reacquire a vertical position, the septal arrangement slowly gives up the bilateral and returns to the radial symmetry. Thus the character on which it was proposed to found a great division of the stony corals was nothing but a slight mechanical adaptation to a passing phase in the life of each individual coral. But it is only fair to say that the whole tendency of recent works on corals has been to discover the invalidity of the supposed division Tetracorallia."

In a previous section (p. 42) I have dwelt upon the significance of bilateral and radial symmetry in corals, and have shown that in both modern and fossil corals the developmental stages throughout are bilateral, and that it is only towards maturity that the most nearly perfect radiality is assumed. It is the order in which the mesenteries and septa appear in corals which gives their distinctive significance to modern and extinct forms, not their bilateral or radial symmetry. Even the first six pairs of mesenteries which arise before any skeleton appears are arranged in a strictly bilateral manner in modern corals, and the subsequent mesenteries and septa also follow the bilateral plan. When fully developed the majority of rugose corals are as perfectly radial as are modern corals. The statement "that the whole tendency of recent work on corals has been to discover the invalidity of the supposed division Tetracorallia" is made on only a partial view of the case. The only recent discovery of any importance in support of Bernard's position is the demonstration, mainly by Miss Ogilvie, of the unity of microscopic structure of the skeleton of rugose and modern corals, a discovery which was to be expected considering that the polyps forming the two groups of skeletons belong to one group, the Zoantharia. Even the discovery by the "Challenger" of the coral *Moseleya*, which, from Quelch's account, was hailed as a living representative of the Tetracoralla, and as breaking down their distinction from living corals, is shown in Bernard's present paper (p. 24) to be altogether unworthy of the importance attached to it, though on grounds which I do not consider the most fundamental. My demonstration that the protoseptal stage of rugose corals is hexameral, like the corresponding stage in modern corals, proves that the Tetracoralla and Hexacoralla are more closely related than has been supposed, even though from this stage they diverge to the same degree as do modern zoanthids and hexactinians. Bernard's assertion is in harmony with others published by Bourne and Ogilvie. In making such these authors have wholly neglected or minimized the significance of the difference in septal sequence between fossil and modern corals, and hitherto it is only such a character which has rendered possible a natural classification of the Anthozoa. Morphologists and systematists know of no character among the Anthozoa of greater value for taxonomic purposes than the mesenterial (and septal) plan. The hexameral protoseptal stage indicates that the tetracorallids and hexacorallids have a common ancestry, but beyond this stage we have no evidence as to their relationships; we have no forms which indicate how the divergence took place, any more than we have for the soft-bodied hexactinians and zoanthids. We cannot think of them as one group derived from the other, but as divergences from a common ancestry.

When this account was practically completed I received three papers from Prof. N. Yakovleff, all devoted to studies of the Rugosa.[1] One, "Ueber die Morphologie und Morphogenie der Rugosa," contains special reference to the subject of the present contribution, and calls for notice. Yakovleff has found that in Russian specimens of *Lophophyllum proliferum* the concave and convex sides of the corallite are the reverse of those in American specimens. In Russian examples the main or ventral-directive septum occurs on the convex side, while in the American it is on the concave side of the coral, the ventral axial fossula varying in like manner. Although in the great majority of rugose corals the ventral-directive septum (Hauptseptum), along with its associated fossula, occurs on the convex border, yet Yakovleff finds the relationship to be occasionally reversed. Such differences the author considers to be a mechanical necessity in the formation of the secondary septa, according as to whether the mouth or oral surface of the living polyp was inclined to one side or the other of the corallite. In the majority of cases it is supposed that the oral disc was inclined towards the concave side, whereas when the ventral directive septum is on the concave side, the polypal disc was directed towards the convex border.

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[1] 1. "Die Fauna der oberen Abtheilung der palæozoischen Ablagerungen im Donez-Bassin. II., Die Korallen," Mém. du Comité Géologique, Nouv. Série, Livr. 12. 1903.

2. "A Contribution to the Characteristic of Corals of the Group Rugosa." *Ann. Mag. Nat. Hist.*, Ser. 7, Vol. XIII., Feb., 1904.

3. "Ueber die Morphologie und Morphogenie der Rugosa," Ver. der Russisch-Kaiserlichen Mineralogischen Gesellschaft zu St. Petersburg, Bd. XLI., Lief. 2, 1904.

From Yakovleff's account it is evident that various authors have been misled as regards the orientation of the corallite by assuming that the concave and convex borders are morphologically the same throughout. As he points out, the only reliable criteria for purposes of orientation are the relationships of the septa among themselves. Though the axial fossula may occur on either the convex or the concave side of the corallite, it is always associated with the cardinal or ventral-directive septum; the ventral border may be either convex or concave, or indeed at any angle to the concavo-convex axis, according to the species or even individual.

Yakovleff investigates rather fully the claim which has been made for the occurrence of three or four fossulæ in certain species. With regard to *Omphyma* he shows that the presence of more than one depression is doubtful, as also in the genus *Menophyllum*. He concludes for the Rugosa in general that only one fossula, the ventral-directive, is really determinable, the others resting upon insufficient evidence. In support of this he refers to Bernard's statement, already quoted, that he fails to see the evidences for the existence of more that one true fossula in any coral.

In connection with this I would say that whether the alar region with its group of smaller septa, and more or less special interspace, be included within the term fossula is merely a matter of definition. In the forms already referred to (p. 44) such special regions among the septa do occur, and correspond with developmental stages of others in which they are absent at maturity. They have been described as fossulæ by all writers hitherto, and the purpose of the present paper is to show the true morphological significance both of them and of the axial fossula. It must be admitted, however, that the axial fossula is the only one corresponding with any structural peculiarity (siphonoglyph) of the mature polyp.

It is also incorrect to say that a fossula never occurs on the dorsal border of the corallite in association with the dorsal-directive septum. As mentioned on p. 42, specimens of *Zaphrentis compressa* occasionally show a marked depression in this region, of the same nature as that on the ventral border.

As would be expected, Yakovleff (p. 408) is unable to accept Bernard's explanation of the fossula as due to a bagging down of the basal tissues consequent on the overturning of the prototheca. He also (p. 400) shows the untenable nature of Bernard's view that the bilaterally symmetrical Rugosa have risen from the radially symmetrical Madreporaria as a result of the falling over of the corallite.

Yakovleff (p. 402) evidently shares the general view that the fossulæ are due to the presence of smaller *primary* septa ; " Die Septalgruben sind Viertiefungen, die sich bei den primären Septen befinden . . . in den Septalgruben zeichnen sich die primären Septen gewöhnlich durch ihre schwache Entwickelung (geringen Dimensionen) aus." I have shown above (p. 32) that though the alar fossulæ are always associated with a group of new smaller metasepta yet the primary alar septa (protosepta) are not smaller than the other primary septa ; they form the ventral limit of the alar fossulæ, but themselves take no part in any depression or enlarged interspace. Only the ventral axial fossula has associated with it a smaller primary septum, and this constitutes its fundamental distinction from the lateral depressions.

SUMMARY.

1. The two alar fossulæ present in certain rugose corals correspond with the region of addition of new septa within the middle two of the six primary interseptal spaces, and each is situated on the dorsal aspect of a ventro-lateral or alar septum. The fossula is due to the fact that some of the septa are here shorter,

and are inclined towards and fused in a successive manner with the dorsal older septa.

2. Alar fossulæ indicate with an incomplete stage in the radial development of septa, and similar stages are passed through in the ontogeny of other rugose corals in which the mature calice attains more nearly radial symmetry.

3. The cardinal or ventral-directive fossula where best developed is formed by two distinct structural elements: (*a*) a group of incompletely developed septa on each side of the ventral directive or cardinal septum, and (*b*) a ventral directive or cardinal septum smaller than the other septa of the first cycle.

4. The two ventral groups of incompletely developed septa have a significance similar to that of the alar fossulæ, that is, they represent a developmental stage.

5. The smaller cardinal septum was probably correlated with the presence in the rugose polyp of a ventral siphonoglyph or gonidial groove in the stomodæum, like that which occurs in the living Zoantheæ.

6. In the most radially developed species the simple cardinal fossula is represented by only the shortened directive septum.

7. Like modern corals and cœlenterates generally all the Rugosa exhibit bilateral symmetry during their development, and as they approach maturity they become more or less radial.

8. The bilaterality and radiality of tetracorallids and hexacorallids are of very different origin and character, and along with other characteristics of the two groups do not imply any relationship beyond the protoseptal stage.

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THE POSTGLACIAL DISPERSAL OF THE NORTH AMERICAN BIOTA.[1]

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[From the University Museum, University of Michigan.]

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1. INTRODUCTION.

During the last few decades there has been a remarkable accumulation of data on the geographical distribution of the extra-tropical North American biota. In addition to the facts themselves there has been a great advance in those allied sciences which throw some of the most important side lights upon distributional problems — the physiographic and geographic histories of definite areas.

It is of interest to know that the leading factors in this increase in our data have been the surveys by our National Government, especially the work of the U. S. Biological Survey, State Surveys and the great activity of our larger museums, although specialists and amateurs must not be overlooked.

While among many groups there have been notable advances, yet for others our knowledge remains very incomplete, and must apparently remain so because of the immensity of the field and the scarcity of workers. Here even the preliminary organization of data is yet to be made. The recent advance then in distributional work has been largely due to the great increase in the amount of data.

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[1] Read at the Eighth International Geographic Congress, Washington, September 9, 1904.

The next general step of advance which we may expect to follow this stage of rapid accumulation of facts is that of their explanation or interpretation. At present this phase of the subject is much confused by the babel of opinions as to the relative importance of various influences controlling distribution. There are several points of view, and each worker is keen to the influence of certain factors. It is to be hoped that this diversity of opinion will lead to a period of discussion, enriched by many suggestions and discoveries of relations, previously unnoticed. Such a period would certainly hasten the correlation and interpretation of much miscellaneous and imperfectly organized data.

It is to one phase of the subject of faunal interpretation, and the dynamic aspect of the historic factor in particular, to which special attention is directed in this paper. That the historic factor is a real one is very generally recognized, and yet in spite of this fact it is difficult to fully realize that the present distribution which we see, is largely an *effect* of past conditions; the cumulative result of many factors, and not controlled altogether by the conditions of the present environment. To properly estimate this factor it becomes necessary to reconstruct the successional relation and the past conditions, and thus see how each stage has prepared the way for the following one. We must reconstruct the past, for this is as essential in geographical distribution as is the restoration, in the mind of the paleontologist, of the soft parts of the fossils he wishes to interpret.

Some phases of this subject are much more simple than others, just as the history of one region may be much simpler than another. From the biological standpoint this is certainly the case with that part of North America repopulated during the decline of the Wisconsin ice sheet.

To fully understand the return movement to the glaciated region, it is necessary to know the time relations of the various Pleistocene deposits of fossils, as during that time there flourished a variety of forms no longer members of our present fauna. The mastodon, mammoth, peccary, camels, tapirs, native horses and many other forms were then abundant. But, as this phase of the subject is unfortunately in a very chaotic state little help can come from this source at present. Yet there are certain facts derived from these fossils which are very significant. For example, the occurrence in Pleistocene times (Hay, '02; Hatcher, '02) of such arctic types as the walrus in Virginia and South Carolina along the Atlantic coast, the musk ox in Pennsylvania, West Virginia, Kentucky, Indian Territory and Iowa, and the reindeer in New Jersey, Pennsylvania, Kentucky and Iowa, certainly shows that an arctic climate once reached far to the south. Although limited, this information clearly suggests the general extreme southern limit reached by the arctic types during the Ice Age. As the Wisconsin Ice Sheet was not the maximum one in southern extension, these arctic types, in their last migration in all probability, did not start from this extreme southern limit, but to the north of it. This gives us an approximate starting point in eastern North America of the Postglacial return of life to the glaciated region. From the Great Plains westward the ice sheet did not extend far to the south in the United States so that the return movement in that region began much farther to the north, near the Canadian boundary. At present, as has been said, paleontological facts do not greatly aid in understanding the early Postglacial northward extension of the biota. But there is another source of information to which we may appeal and that is to the affinities or relationships of the biota south of the ice margin. This makes it necessary to take into account the general conditions of life in North America south of the Wisconsin ice margin, and hence to the areas of preservation which must have existed in North America during the Ice Age.

2. Biotic Preserves During the Ice Age.

Repeated glaciation had almost sterilized the northern part of the continent. From whence then came the life now occupying that region? Many of the problems involved in a reply to this question cannot be answered at present, but others may be, in an approximate manner. Much exploration remains to be done in northern Asia before we can hope to answer certain questions on those elements in our fauna and flora which have decided Asiatic affinities. But when we consider the more characteristic

American elements, a much greater degree of definiteness may be secured. From our knowledge of the distribution and conditions of life of the present biota, it seems that while the northern part of the continent lay deeply buried under the mantle of the Wisconsin ice sheet there existed, in all probability, south of the ice margin three distinct belts of life (Adams, '02, *b*). At or near the ice margin, and perhaps only forming a narrow transcontinental belt, was the tundral or barren ground biota. Below this first came stunted trees and shrubs, and farther back coniferous forests forming a transcontinental belt, but composed of two distinct types, an eastern and a western one. Below this, in turn, came a third belt of still less homogeneity; in the east it was composed of deciduous forests and their associated fauna, while in the west it was made up of plains and desert types of life.

With these conditions in mind let us now turn to a more detailed consideration of the various elements which go to make up these belts of life, and attempt to follow some of the dynamic phases which this biota has shown since Glacial times. The fundamental idea in following these dynamic changes is that we have belts of physical conditions migrating to the north. Thus there has been given a definite trend to the environment. This fact cannot receive too much emphasis. Just as when studying the littoral fauna of a pond or lake it becomes necessary to bear in mind the dynamic tendency of this littoral zone — that is for it to move inward toward the deeper water — so in a similar manner, to understand the dynamic changes in life areas or zones we must bear in mind the dynamic tendencies in such areas (Adams, '02, *a*, p. 126). Nor is this limited to climatic and topographic influences alone; it includes organic factors as well. It is also necessary to keep such dynamic tendencies in mind when attempting to follow the relations and movements of these three belts in their Postglacial migrations.

The three belts or waves just mentioned were composed of such distinct elements and have had such varied histories that they demand separate treatment. The wanderings of these different types, since Glacial times, is likely to make the application of geographic names confusing (Fig. 1). The members of the first zone or wave (tundral type) have spread from the Ohio valley to the Arctic sea, the members of the second wave have moved from much the same southern limit to Hudson Bay, but

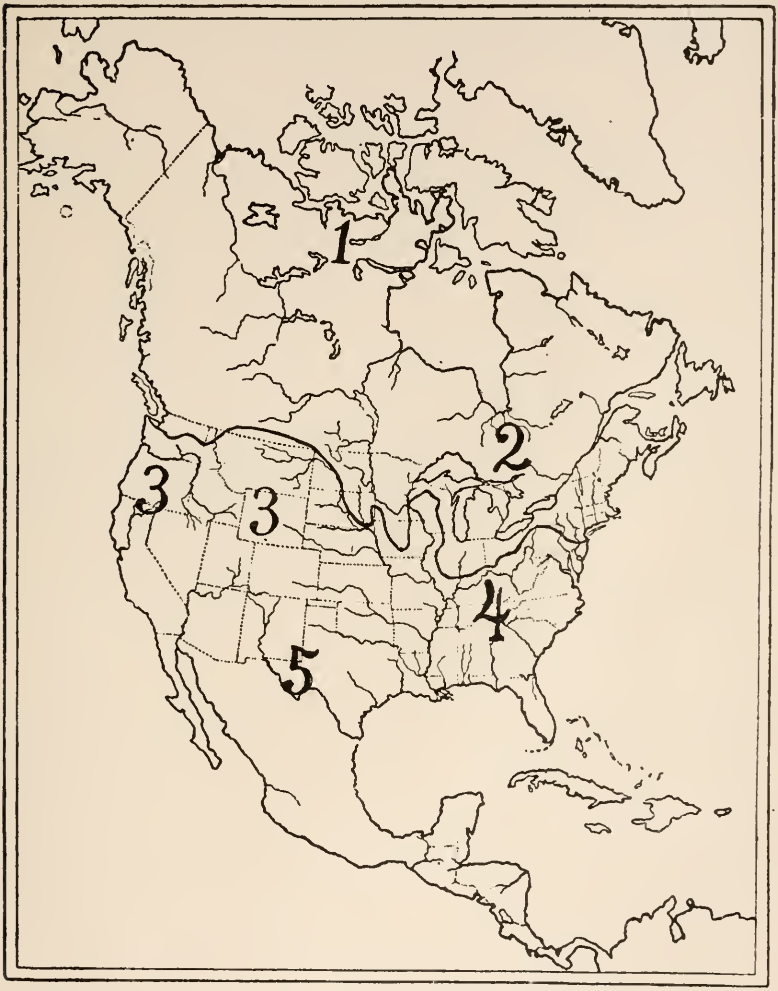

FIG. 1. Showing the present location of the five biotic types and the area invaded by them in Postglacial times. The transverse lines indicates the southern margin of the last (Wisconsin) ice sheet.

the third wave, composed of the southeastern and southwestern biota, has been relatively stationary. The first two waves entered the territory they now occupy largely from the *south*, although we usually think of them as completely boreal forms and tending southward in their dispersal. Turning now to a brief consideration of the primary characteristics of these elements in the biota and beginning with the one which invaded the glaciated region first we have the following order of succession :

First Wave.

1. *Tundral or Barren Ground Biota.* — This element of the return movement exists to-day in the north beyond the tree limit and as relicts farther south upon mountain summits. This is a circumpolar type and has little peculiarly American. Its original center of dispersal may have been farther south near the centers of ice accumulation, or as Dixon ('95, p. 298) has suggested in the elevated regions of the tropics. In either case it has had a very nomadic existence.

As there is reason to believe that the ice did not completely cover all the northern land some of this type flourished there, in all probability, even in Glacial times, as for example, in the Point Barrow region of Alaska, where Nelson ('87, p. 27) has noted the distinctly Siberian affinities of the biota. This factor suggests that the life of this region is an overflow perhaps in preglacial or Glacial times from unglaciated Arctic Siberia.

Migration and Dispersal Routes. — The first wave biota has apparently reached its present location by a northward Glacial and Postglacial migration, and has been supplemented by certain Glacial relicts, from Alaska in particular, as has been suggested, while in more recent times some additions have been received from Asia and Greenland as has been shown by Stejneger for the wheatear ('01, *a* and *b*). The migration route of the western birds of this species points to India by way of Alaska, and the eastern ones to Africa by way of Greenland.

Probably the latest paths followed northward were along the mountain chains, where occasionally colonies have lingered, in favorable conditions, upon mountain peaks.

Second Wave.

2. *Northeastern Biota.* — The second wave was of the biotic type now represented by the northern transcontinental coniferous

forest belt. But this belt was not homogeneous from east to west and the eastern element in this wave will be considered first. On account of its wanderings, this wave as in the case of the first cannot be geographically defined in the east as a center of dispersal, without danger of confusion. Although this biota reaches its best development at the present time, in the northeast, yet it is only a relatively late arrival in that region. For eastern North America this was the "second wave" (Adams ,'02, *b*, p. 309) to pass north after the retreating arctic climate attending the decline of the Wisconsin ice sheet. The region now occupied by this biota is an area with abundant lakes, peat bogs and a region of poor drainage. The area is covered by coniferous forests but it is of a very different type from that found in the Rocky Mountain region as has been shown by Rydberg ('00, pp. 871–873). Here the very characteristic bog plant society reaches its best development as shown by Transeau ('04). This is the region of fur-bearing animals, and there are very few reptiles and amphibians. On the west this biota swings north of the Great Plains in Canada to the Rocky Mountains and then north into the Mackenzie basin.

Migration and Dispersal Routes. — The northeastern type of biota has moved from about the latitude of the Ohio valley north to its present position. Certain elements have apparently pushed far northwest to the Rocky Mountains, to the Mackenzie basin, and even overflowed into the Yukon valley — the reverse route, in all probability, was followed by certain Asiatic forms into America. This westward and northwestward dispersal has tended perhaps to overemphasize the transcontinental distribution of these northern types and shows how the determination of faunal areas based primarily upon the present conditions tends to obscure the compositeness and diversity of origin of their constituent elements. This biota reaches its greatest southward extension along the Appalachian Mountains. Laggards of this and the barren ground type form the " boreal " islands, when surrounded by the life of the following wave. These occur not only upon mountain tops, in bogs and on sand dunes but also in certain deep lakes, where the " abyssal " fauna shows very decided northern affinities, and clearly suggests them as Glacial relicts.

3. *Western Center of Dispersal.* — In the West we recognize a second center of northward migration. It is represented by the biota of the Rocky Mountains and the Pacific coast region. Its great extent, even in Glacial times, south of the ice margin and its present occupation of the field, allows this biota to be geographically defined as the Western Center of Dispersal. In contrast with the region dominated by the eastern part of this wave the western branch occupied a high mountain country. It was a coniferous forest belt, but as has been mentioned, was of a very different type from that of the northeast. The present flora of the Rocky Mountains and the coast region is of the same general type, as shown by Coville ('93, pp. 29–31) and Rydberg ('00, p. 871), although the climatic conditions are quite different in several respects. It should also be recalled that much of the recent botanical work has been done in the Rocky Mountains near the Canadian border so that later studies in the southern Rockies may, to some degree, lessen this apparent uniformity. These facts do not favor the idea of transcontinental unity of the coniferous forests but show that the direction of geographic origin, the adaptations of the biota to mountain conditions, and proximity, are factors which must be reckoned with in understanding the Postglacial repopulation of the Northwest. The same factors also suggest that the usually accepted transcontinental distribution of the fauna may be overestimated. At least it is very evident that many of the characteristic animals of the western mountains are lacking in the relatively low eastern Appalachians. Such a relation may have been closer in the past than it is at present, as is suggested by the occurrence of the pica (*Ochotona*) in the Pleistocene of Pennsylvania, although now, in North America, it occurs only in the western mountains. There is also in the West a great increase of Asiatic types, in addition to certain native elements. The mountain goat (*Oreamnus*) and big horn (*Ovis*) are representative mountain forms of the west but lacking, even as fossils, in the east. Among some invertebrates this is also true, as for example, the butterfly genus *Parnassius*, the crawfish *Potamobius*, the west coast *Unionidæ* and certain *Arionta*-like land shells, are quite distinct from eastern types. The composition and affinities of this western biota

require much more study before its true position can be determined. In marked contrast with the northeastern biota this one has long been bounded on the south and east by an arid climate.

Migration and Dispersal Routes. — With the exception perhaps of Glacial relict colonies in favorable spots along the Pacific coast, and in the unglaciated parts of Alaska, the biota of the Pacific coast and Canadian Rockies must have pushed into this region primarily from two directions in Postglacial times. To a limited extent there was an overflow of the northeastern biota but the great bulk of the population came from the Rocky Mountains and Pacific coast region south of the Canadian border. Dispersal must have been carried on under great disadvantages, on account of the topographic difficulties. But this biota, on account of its proximity, and early invasion of the region, had manifest advantages over the later arrivals. That a great wave of life moved north along the mountains from this western center is very apparent from the present affinities of the life of the region extending from southern British Columbia to Alaska. The primary highways were probably the mountains themselves, and a narrow coastal strip, now largely submerged. These lines of dispersal are today migration routes for birds. Bishop ('00, p. 50) has shown that a large part of the Yukon valley birds winter in western United States, and this clearly suggests their western origin.

The extensive distribution of certain forms in northwest North America, and their occurrence as well in the northeast, has suggested the northwestern origin of such forms. From the present standpoint it seems more likely that most of these northwestern forms have been derived from the western center of dispersal from which they spread north or later overflowed into the northeast. It also seems that the northern biota in general has had a northern rather than a southern trend to its dispersal.

The Alaskan region, in addition to its Glacial relicts, was apparently repopulated in part by a northward invasion from the western center of dispersal along the mountains, by a double invasion from Asia (Stejneger) north and south of the Stanovoj Mountains, and by contributions from the northeastern biota.

It also appears that some of the mountain biota of the Canadian Rockies were driven north into unglaciated Alaska as the ice spread from the Cordilleran center of ice accumulation.

Third Wave.

4. *Southeastern Center of Dispersal.* — The region occupied by the southeastern type of biota was largely south of the territory invaded by the ice, and its biota has therefore been relatively stable in its geographic position when compared with the extensive migrations of the first and second waves. As today, during Glacial times, this biota was bounded on the west by the arid plains. This is a region of low plains and plateaus, the higher mountains within this area still retaining the second wave types as Glacial relicts. It is probable that the first wave type never reached in abundance so far south. The climate of this southeastern center is equable and there is abundant rainfall. The dense deciduous forests furnish favorable conditions for animal life. This region has not only been important as a region of preservation, but also as a center of origin. Here there is the best development of the deciduous forest and the most characteristic features of the land and fresh water shell life of North America. This has also been the center of distribution of several vertebrate types and also for certain plants. But as this center has been discussed elsewhere (Adams, '02, *a*) only brief mention will be made here of its characteristic features.

Migration and Dispersal Routes. — With the retreat of the ice this biota formed the eastern element of the third wave. It moved north and northwest behind the coniferous forest zone. But as this biota was relatively stable its center of dispersal can be definitely defined as occupying southeastern United States, east of the Great Plains. This stability therefore makes the dispersal routes of more importance than the migrations of the biota as a whole, as the spread of this biota has apparently been influenced more by the normal increase of a populated area than by a great change in the physical conditions which was such a dominant factor farther north.

The primary routes fort he land forms were the Coastal Plain and its valleys, the Appalachian plateaus, and the Mississippi and tributary valleys. For the aquatic types the Tennessee and Mississippi Rivers were the leading highways. From the upper end of the Coastal Plain a limited number of land forms pushed up the Hudson and even worked west via the Mohawk valley to the Great Lake region. From the Mississippi numerous tributary valleys were followed, the Ohio, Wabash, Illinois, and Missouri, and thus this biota radiated rapidly. It even invaded the Great Plains along eastward flowing streams, especially along the Missouri River.

The second wave types reach their most southern extension along mountains while this third wave reaches its most northern extension along valleys, not only to the north but also upon the arid plains of the northwest.

5. *The Southwestern Center of Dispersal.* — The area occupied by the southwestern biota was largely far beyond the ice margin and, like that of the southeastern, was relatively stable in its geographic position. At present this type is represented by the life of the arid southwest, including the Great Plains, the Great Basin, the central valley of California and the Mexican Plateau. It is a vast region of arid plains, desert plateaus, and mountains, subject to great climatic extremes. In spite of the severity of the conditions of life the biota is quite varied, and many kinds are abundant. Attention has already been called (Adams, '02, *b*, p. 121) to the importance of this center and too much emphasis cannot be placed upon its importance, not only as a center of distribution, but also as the region of origin of the arid North American biota. It seems equally evident that before a reliable estimate can be made of this biota it must be carefully compared with that of the arid regions of South America and of Asia. The life of the first and second waves in the Postglacial migrations contained many forms not peculiarly American, but the southeastern and southwestern elements of the third wave show much more individuality. The southeastern center has certain endemic elements in its flora and fauna, yet several other types have their affinities duplicated in eastern Asia, and thus its individuality is somewhat lessened. On the other hand the southwestern center, although it shows some Asiatic duplication, does not appear to be so marked. So far as known to me no one has

made a detailed comparison of the arid types of the two continents.

The distinctness of the southeastern and the southwestern centers is frequently overlooked or confused. And this is especially liable to be the case when allowance is not made for the influence of local conditions upon the occurrence of certain southwestern types which have overflowed into the eastern center. This brings up the following question, which as will be seen later on, clearly emphasizes the importance of habitat study in geographical distribution. In estimating biotic areas, how much weight should be given to the occurrence of forms dependent upon limited local conditions? A bare census gives no idea of the relative weight of the units recorded or the degree of representativeness of them. The importance of such a study in a proper estimate of local conditions has been suggested repeatedly in attempting to determine the relations of these two centers. These relations have suggested that perhaps biotic affinities can be more easily and safely determined by habitat and biotic associations than primarily upon a faunistic or floristic basis. This would mean that the ecological relations rather than the taxonomic affinities should receive greater attention than is customary. It should be noted, however, that this view does not in any way belittle the importance of taxonomic work in distributional studies. But it is sufficient, at this place, simply to call attention to the ecological aspect of the subject.

But to return to the consideration of the southwest, the vegetation of this arid region is composed of grasses on the plains, and cacti, agave, yucca, and many other types of desert vegetation in the more arid places. Reference need only be made to the recent paper of Coville and MacDougal ('03) for the characteristic features and the literature on this flora. The fauna is equally peculiar and interesting. This is the region where prairie dogs, spermophiles, pocket gophers, pocket mice, wood rats, kangaroo rats are so characteristic, and the horned toads, rattlesnakes and many other reptiles reach their greatest variety and center of abundance. This has been the center for many other forms as well. Certain crawfishes (Ortmann, '02) have originated here. Many groups of insects are also characteristic. The bees of the genus

Perdita are very abundant, and as Prof. T. D. A. Cockerell informs me, are very characteristic, only a few species occurring east of the Great Plains (Cockerell, '98). The beetles of the family *Tenebrionidæ* are quite abundant. The ant-lions, *Myrmeleonidæ*, here reach their greatest development in variety and abundance. The fish fauna is limited and peculiar ; those of the Rio Grande have Mississippi River affinities, while those of the Colorado River show much endemism, as shown by Meek ('03). Of its 32 species, only 10 are known to occur elsewhere.

Migration and Dispersal Routes. — As only a small part of this southwestern center was invaded by the Glacial ice its geographic position has been relatively stationary. Since the Ice Age, however, there has been considerable overflow to the north. Starting in the southwest this biota has been spread northward along each side of the Rocky Mountains, and has invaded an arid region, a condition to which it was evidently well adapted. Even glaciated portions of British America were reached on each side of the mountains by these hardy forms of life.

Other plants and animals have spread from here into the southeast, where on account of its varied conditions of life they have been able to flourish. This, for example, is seen in the case of yucca, lizards, and pocket gophers. These forms have been able to find favorable arid *local* conditions in the southeast, as in the pine barrens and on dry hillsides, etc.

These arid types find their eastern extensions upon the dry uplands interdigitating with the southeastern types which frequent the moist valleys. They reach their extreme eastern extension, in abundance and in association, upon the prairies of Wisconsin, Illinois, and northern Indiana. But with the clearing away of the forests this eastward advance has been greatly hastened.

The aquatic life of this center has communicated with the Mississippi River, as shown by the Rio Grande fish fauna, but that of the Colorado River has been isolated to an exceptional degree, and has developed a remarkable individuality.

3. Some Factors in Biotic Interpretation.

In a previous paper (Adams, '01) the writer has discussed the relation of the baseleveling processes upon habitat differentiation and their influence upon the successional relation of the faunas correlated with the degree of topographic development of a region. Although the fauna was mentioned in particular, these factors influence the entire biota in a similar manner. During the process of degradation of the land there is a definite and orderly succession of conditions through which the habitats pass ; the brooks become larger streams, the lakes and ponds become drained, and the uplands are lowered, etc. Not only does the location of the habitats change but also their relative positions and extent. On account of the great influence which topographic conditions have upon habitats it is possible to find very diverse biotic conditions even in a relatively small area. Students of local faunas and floras frequently comment upon this diversity, and although these facts are often noted, yet but little attention is given to them because of their seeming chaos. This apparent mixture or confusion is often due to a total disregard of the habitats and the associations of the forms in them. That this occurrence is, as a rule, quite definite and orderly, may be seen by reference to the example of certain southeastern types evidently of western or southwestern origin, the yuccas and pocket gophers. These are types from an arid region, and it is important to note that when they invade a moist region they occupy the relatively dry situations — the pine barrens, sandy or rocky places — for such are the conditions most nearly approaching their original home. Such colonies form " islands " of arid types surrounded by those correlated with greater moisture. The significant fact here is the definiteness of the conditions in which they occur. Again this same tendency is shown in the extreme northward extension of the southeastern biota along protected valleys, and even far out upon the Great Plains. Similarly in southern Michigan, certain characteristic members of the southeastern biota enter the state at the southeastern and southwestern corners, rather than along the southern border, because valley highways enter the state at these corners. Apparently this same route into southeastern Michigan has been utilized by certain forest trees, insects, birds and doubtless other southern types, which have also invaded extreme southwestern Ontario. Such facts might be indefinitely multiplied, but these clearly show that invading *elements tend to enter a region, not only at a definite place, but also tend to remain in definite habitat associations and conditions even after having once entered a region.* This habitat individuality causes more or less isolation of the various elements invading a region and furnishes an index to their direction of origin, and at the same time reinforces the idea of the regularity of their field relations. To be sure this definiteness becomes more or less blurred and indefinite along tension lines, but it is not confusing when considered with the proper perspective.

It is quite evident that the kinds of biota frequenting similar habitats must be largely different in distinct biotic regions. This may be seen by a comparison of the same habitats in regions occupied by distinct biotic types. Thus if a comparison is made between the shell life frequenting the margin of an isolated pond in Michigan and that occupying the similar habitat of a sink hole pond in east Tennessee, a marked dissimilarity is noticed. In the northern pond there will be an abundance of shells belonging to the genera *Limnæa* and *Physa*, while in the southern one these genera will be poorly represented or entirely absent. If a similar comparison is made between the shells found in rapidly flowing brooks, from the same regions, the southern stream will abound in shells of the family Pleuroceridæ, a family poorly represented in the north. The Limnæidæ are northern in their distribution, and the Pleuroceridæ are characteristically southeastern. The same general relations hold for the vegetation ; in the southeast there is the deciduous forest and in the northeast a coniferous one.

On account of the unique character of the life occupying the same kind of habitats in distinct biotic regions, there results, in these regions, a different succession of forms attending changes in the topography, climate or any other factors which may influence habitats. *Thus the succession of ecological associations is likely to be similar, even in very distinct regions when similar processes and conditions are at work, yet the biotic components, the families, genera or species, etc., are likely to be quite different.* Such relations as these mean that from very diverse kinds of life there tends to be formed, *de novo* or by association, certain ecological types which become correlated with certain habitats.

Thus certain habitat types have originated many times independently. For example, the fresh water fauna was not formed all at once. This environment has been independently and repeatedly invaded by very diverse animals and from diverse habitats. The same is equally true of the minor fresh water habitats, such as that of the littoral zone or the rapid water of a brook, etc. The same is equally true of land habitats, such as caves, deserts and many other situations whose biota has been derived from all possible directions. It is thus evident that there are two fairly distinct classes of succession in a given biotic region, the adaptational one, in which the ecological aspect is prominent, and the hereditary one, in which the taxonomic or hereditary aspect receives emphasis.

From the above considerations of habitats and their biota, their successional relations and their convergent habitat and ecological tendencies, we are led to a very natural question : What is their bearing upon migration and dispersal centers ? This relation is very close and unfortunately only too often completely overlooked. If in the study of the life of a given region practical recognition can be made of the above mentioned relations, which are involved in the study of the origin of the biota of given habitats, there will result a very desirable *geographic perspective*. Such a perspective will greatly aid in the determination of the relative influence of the factors of the environment. As the habitats of many plants and animals change with geographic range it is very desirable to take advantage of this variation in estimating the relative influence of different elements in the environment. In this way we may hope to distinguish between the local and geographic conditions, influences which are easily confused. It is primarily their reflex effect upon geographic distribution, and especially upon biotic interpretation which interests us at this time, a subject which can not be separated from a consideration of the relative influence of environmental factors. Habitat studies not only throw important light upon geographic origin of biotic elements but also upon the conditions of life determining routes of dispersal, and these are often very important elements in biotic interpretation. For it seems at present that it is along this line that we may expect, in the near future, some of the most rapid advances in distributional problems.

4. SUMMARY AND CONCLUSION.

In summarizing we may note that recent advances made in the study of the extratropical North American fauna and flora have been primarily due to the rapid accumulation of data. In the near future, rapid advances along the line of explanation and interpretation of these facts may be expected. As the present distribution is in part an *effect* it is therefore necessary to take into account certain past conditions. Very important among these factors have been Glacial and Postglacial influences upon this region. These geological changes have had a great influence on the biota, not only on account of the wonderful changes in the physical conditions of life, attending the decline of the Ice Age, but also on account of the definiteness given to the dynamic tendencies by this environment. When attempting to determine the affinities and interrelations of the present biota too much emphasis cannot be placed upon this definite dynamic tendency, and to the sources and routes followed by the life on its return to the glaciated region. This returning biota followed, in all probability, a definite successional relation and was composed of three general belts or "waves," concentrically distributed south of the ice margin. The first one was of the barren ground type, the second was represented by distinct eastern and western coniferous forest types, and the third by the biota of the southeastern and southwestern states. The first wave was of a transcontinental extent, the second while coniferous and transcontinental was composed of two distinct types, the eastern, represented by the biota of northeastern North America, and the western by that of the Rocky Mountains and the Pacific coast. The northeastern biota overflowed to the north, to the northwest into the Mackenzie basin and even a few forms into the Yukon valley and to the Rocky Mountains. The northwestern biota spread from the Rocky Mountains and Pacific coast regions in the United States north to British Columbia and Alaska. The third wave spread from the southeastern center of dispersal northward to the conifers, and west to the Great Plains. From the southwestern center the life spread north on each side of the Rocky Mountains into Canada, and only stragglers spread eastward into the humid southeast.

Further light is to be thrown upon the interpretation of these centers of dispersal and their biotic types by taking into account the successional relation of the biota, as correlated with changes of the environment. The habitat relations of organisms show that they do not occur promiscuously mixed, even within a small area, but that their relations are orderly and definite. In addition to the general successional relation attending changes of the environment, attention is called to the different kinds of organisms in different biotic regions, which make up this succession. This habitat uniqueness of the biota in different regions favors the independent formation or association of similar habitat types from very diverse kinds of biota.

With these sources of Postglacial supply, their routes of dispersal, and their definite habitat relations fresh in mind, it becomes very evident that these factors must greatly influence our interpretation of life areas. These facts strongly suggest that the present conditions of life cannot be expected to fully explain the present distribution, and clearly emphasize that the historical factor must be dynamically considered.

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BIOLOGICAL BULLETIN

THE FORMATION OF THE FIRST POLAR SPINDLE IN THE EGG OF BUFO LENTIGINOSUS.

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In a previous paper on "The Maturation and Fertilization of the Egg of *Bufo lentiginosus*" (King, 10), the formation of the first polar spindle and the subsequent divisions of the chromosomes were very incompletely described owing to a lack of material showing the details of these processes. During the spring of 1899, a large number of toads were collected soon after they had emerged from their hibernation, and from three of them sufficient material was obtained to give a more complete history of the late maturation processes. A short account of my study of this period in the development of the egg has already appeared (11); a detailed account is given in the present paper.

I. Material and Methods.

As soon as possible after the toads were captured they were killed by pithing and the body opened at once to ascertain the condition of the ovaries. In a great majority of cases the eggs were found free in the cœlomic cavity and were, therefore, of no use for the purpose intended, as previous investigations had shown that eggs which have broken through the wall of the ovary invariably contain a fully formed maturation spindle lying at the periphery near the center of the black hemisphere.

In several instances, all of the eggs were still attached to the walls of the ovaries when the toad was killed. In these cases some of the eggs were put at once into a dish of fresh spring water and the rest were left in the body of the female which was kept in a moist chamber. A few eggs from each of these series were then fixed at intervals of ten minutes for a period of several hours. By opening an egg under a dissecting lens after it has been taken from the fixing solution and put into 50 per cent. alcohol, one can tell definitely whether the late maturation processes have begun or not; for, if the nuclear membrane in still intact, the nucleus retains its rounded form and can be readily separated from the rest of the egg contents. If one hour after the toad is killed, an examination of freshly fixed eggs shows that the nucleus is still intact, the entire set of eggs can be discarded, as it has been found that further development does not take place unless the germinal vesicle breaks down previous to this time, although the eggs, whether kept in water or in the body of the female, show no signs of disintegration for many hours.

In one case the germinal vesicle was just breaking down when the eggs were first examined under the dissecting lens; in another set of eggs the germinal vesicle could no longer be dissected out half an hour after the toad was killed. These two lots of eggs gave overlapping series of stages which corresponded in every respect. A third set of eggs showed no signs of the germinal vesicle when first examined, and when sectioned showed maturation processes identical with those taking place in eggs which had been developing in water for several hours.

In all these three sets of eggs, the first polar body was given off in the normal position and apparently in the normal manner before the eggs showed any signs of disintegration. No difference was noticed in the development of eggs which had been put into water and those which had been left in the body of the toad. It does not seem, therefore, that such unusual conditions interfere at all with the late maturation processes provided these processes have started before the normal conditions are changed. No attempt was made to fertilize these eggs artificially, as it has never been found possible to fertilize either the eggs of *Bufo* or of *Rana* until they have received the thick jelly-like membrane which is secreted around them in the oviducts.

In all cases the eggs were fixed in corrosive-acetic and stained with a combination stain of borax carmine and Lyon's blue as described in a previous paper (King, 10).

II. The Disintegration of the Germinal Vesicle.

I have already given in detail a description of the early stages in the breaking down of the germinal vesicle, and as this new material confirms but adds nothing to that description, it will be necessary to give only a brief account of the changes in the egg directly preceding the formation of the spindle.

At the end of the hibernation period the germinal vesicle lies in the upper hemisphere of the egg. It is round in outline and contains a large number of nucleoli which usually form a ring enclosing the chromatin threads. A layer of granular substance staining differently from the cytoplasm, surrounds the lower pole of the germinal vesicle and extends half way up each side. This substance appears homogeneous at first and then becomes a compact, fibrous band of uniform thickness. I have called this band a "line of radiation," because, as soon as the nuclear membrane has disappeared in this region, the karyoplasm of the nucleus forms into coarse granules and a pronounced radiation extends up into the nuclear substance from the entire length of the fibrous band below. The karyoplasmic granules soon become smaller and more numerous and finally disappear entirely, while the radiation from below continues to increase and often extends nearly to the upper surface of the egg. The rays forming this radiation are very fine, and their outer ends run, apparently, into the coarse network which comes to fill the entire space formally occupied by the germinal vesicle. During these changes, the nucleoli have lost their power of staining and have begun to disintegrate.

When the nuclear membrane breaks down, twenty-four chromosomes, arranged in pairs, are scattered throughout the upper part of the nuclear space. The ends of each pair then unite to form a closed ring near which a small aster usually appears. The aster has no centrosome and its rays rarely touch the chromatin ring. At the next stage, when the radiation from below has reached its greatest extent, the asters and the chromatin rings entirely disappear. Later, when the radiation has begun to decrease, a large number of small round chromatin granules are found near or on the line of radiation which has been gradually shortening during this period. When the chromatin granules first appear they stain very faintly, but they soon take the deep carmine stain characteristic of chromatin, and then fuse into several large, irregular clumps.

III. The Formation of the First Polar Spindle.

The line of radiation, shortly after the appearance of the chromatin granules, is shown in Fig. 1. It is a short, fibrous band with its ends, usually, though not invariably, slightly curved in towards the center of the egg. This structure, which is to become the first polar spindle, lies some distance below the surface of the egg in a small accumulation of granular substance formed, possibly, from the karyoplasm of the germinal vesicle. Its longitudinal axis may be either parallel or oblique to the surface of the egg, the latter position being the more common. Running out in every direction from the compact meshwork of fibers are numerous fine, thread-like rays which are longest and most numerous at the middle of the forming spindle where they extend out between the yolk spherules and seem to be continuous with the cytoplasmic network of the egg.

Collected near the middle of the spindle is a mass of small chromatin granules which are of uniform size and stain but faintly in comparison with the chromosomes of an earlier and of a later period. There is a very large number of these granules and it is quite impossible to count them satisfactorily; two other sections of the same egg each show as many granules as are shown in Fig. 1.

The nucleoli from the germinal vesicle appear at this period as irregular, yellowish green, refractive bodies which are scattered throughout the upper hemisphere of the egg, often lying quite close to the spindle. They disappear at different times in different eggs. Sometimes they have all been absorbed before the chromosomes have divided; sometimes they can still be found after the first polar body has been given off. I have never found any traces of them, however, after the spermatozoön has entered the egg.

Not more than fifteen minutes after the stage of Fig. 1, the chromatin granules begin to fuse into irregular-shaped clumps. The number and size of these clumps vary greatly in different eggs, in some cases there are but four or five of them, in others at least twenty. Owing, probably, to their greater volume, these larger masses always stain much more intensely than do the small granules. Meanwhile the spindle has lost its uniform diameter and has become much thicker in the middle where the meshwork of fibers appears more distinct and more regular. The spindle soon becomes barrel-shaped and its fibers are quite clearly defined in the middle region but not at the poles (Fig. 2). The radiation from the spindle disappears entirely except at the poles where it forms distinct asters; some of the rays are very long and cross each other at the equator of the spindle. During its migration towards the upper pole of the egg the spindle shortens somewhat and gradually becomes more slender and pointed, a phenomenon seen by Van Name (17) in the eggs of Planarians, by Korschelt (12) in *Ophryotrocha*, by Griffin (8) in *Thalassema*, and by Boveri (1) in *Ascaris.*

At no stage in the formation of the spindle or in its later history can any centrosome be found in the polar asters. As the spindle becomes more pointed, the rays converge more sharply at the poles, but even when the radial systems are best developed (Figs. 2, 3), the rays appear to run into each other in the center of each aster and there is not the slightest trace of any kind of a central body. Carnoy and Lebrun (2) in their study of the batrachian egg, Eismond (5) in his work on *Siredon* and *Triton*, Fick (6) in studying the maturation of the Axolotl egg, and *Sobotta* (15, 16) in working on the egg of the mouse and of *Amphioxus*, have all failed to find a centrosome in the asters of the polar spindles. If such a structure is normal in these eggs and also in the egg of *Bufo lentiginosus*, methods of fixation and staining which have so clearly demonstrated its presence in other eggs are totally inadequate in these cases to show the slightest trace of it.

At the stage of Fig. 3, the small chromatin granules have entirely disappeared. Whether they have all gone into the large chromatin clumps or whether some have been absorbed by the cytoplasm cannot be determined. At this time the number of large chromatin masses still varies slightly in different eggs; in some cases there are nine such clumps of chromatin, in others at least fifteen. These chromatin masses are, for the most part, scattered irregularly along the spindle fibers, occasionally, however, one or more of them can be seen entirely outside of the spindle (Fig. 4, *CM*). Isolated masses of chromatin are sometimes found near the spindle at a much later period when the chromosomes are at the equator preparing to divide. They have entirely disappeared by the time the first polar body is given off, possibly serving as food for the cytoplasm as suggested by Gardiner (7).

During the next half-hour, the irregular chromatin masses change into chromosomes with a definite shape. The change does not take place at the same time in all of the chromatin clumps; in fact, until the chromosomes are arranged at the equator of the spindle ready to divide, they may be found in several different stages of development on the same spindle. Twelve chromosomes, one-half the number characteristic of the somatic cells of this species differentiate from the chromatin masses. The chromosomes are scattered over the entire spindle and are at first somewhat triangular in shape (Fig. 3), later they become rod-shaped structures which may lie with their long axis parallel, oblique, or even at right angles to the longitudinal axis of the spindle (Fig. 4). Sooner or later, however, the long axis of each chromosome comes to lie parallel with the spindle fibers and the chromosomes then have a rounded knob in the middle region and frequently also a smaller knob at each end (Figs. 4, 5). Later the middle knob becomes more prominent and the end knobs disappear (Fig. 5).

At the stage of Figs. 2–3 the asters at the spindle poles reach their greatest development. There are many long rays from each aster which run nearly parallel with the spindle fibers and cross each other at the equator of the spindle, and fewer and much shorter rays going out in other directions. Soon after this time the asters begin to degenerate. The shorter rays disappear first and by the time the spindle has reached the periphery of the egg there is not a trace of the radiation left. The spindle fibers then converge at the poles which are surrounded by a small accumulation of granular substance probably formed from the disintegrated rays (Fig. 7).

There is often a marked difference in the size of the chromosomes on the same spindle even when they are of exactly the same shape. One or two of the chromosomes may extend over one-third the length of the spindle, the others being not more than one-half as large (Fig. 5). This difference is not found at a later period; for, when the chromosomes are arranged at the equator of the spindle ready to divide, they are considerably smaller than the chromosomes of an earlier period and are all, apparently, of the same size.

While the chromosomes are being arranged at the equator of the spindle they undergo further changes in form. The polar arms shorten considerably, while the thick knob at the middle increases in size and gradually spreads out laterally, thus forming two wing-like projections on the chromosomes (Figs. 6, 7). In proportion as the lateral wings grow larger the polar arms of the chromosomes become shorter and thinner, so that there can be no question but that this lateral growth takes place at the expense of the rest of the chromosome. In a dorsal view, the wings appear to be spread out flat on the spindle and the chromosome has the appearance of a cross in which the polar arms are somewhat longer than the equatorial arms (Fig. 6). In a lateral view, however, the wings are seen to be raised up from the spindle while the polar arms are extended along the spindle fibers. Carnoy and Lebrun (2) have applied the term "oiselet" to this stage in the development of the chromosome. The typical oiselet stage is followed by one in which the body of the "bird" gradually disappears while the wings constantly increase in size (Fig. 7). Very soon, all that is left of the original polar arms is a slight projection on each side of the angle formed by the meeting of the two wings (Fig. 8). In the succeeding stage every trace of the polar arms has disappeared and there are twelve broad V-shaped chromosomes arranged at the equatorial plate with the angle of the V turned in towards the center of the spindle (Fig. 9). Usually, before this last stage is reached, the spindle has come to lie close to the surface of the egg and nearly radial in position. This is by no means invariably the case, however, as sometimes the spindle is still some distance below the surface of the egg when the chromosomes have divided in preparation for the first maturation division.

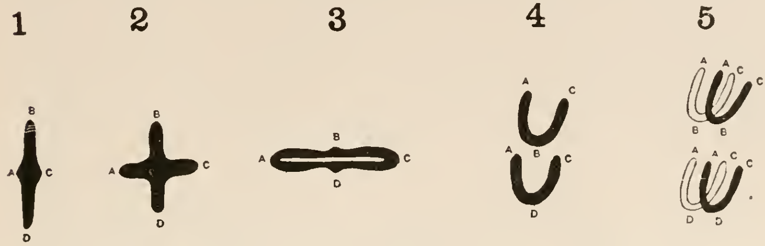
Fig. 6 shows part of a section of an egg fixed as soon as possible after the toad was killed. The spindle lies at the periphery of the egg and the chromosomes, with well-developed lateral wings, are at the equator. That this egg and others from the same series are normal cannot be questioned. They show phenomena exactly similar to those seen in eggs that have been developing in water for some three hours, and leave no doubt but that the earlier processes described above are normal in spite of the unusual conditions to which many of the eggs were subjected.

Four V-shaped chromosomes in which all traces of the polar arms have disappeared are shown in Fig. 9. The arms of the V's are broad flat plates which form a sharp acute angle with each other. There is, in this case, no sign of a splitting in any of the chromosomes which are all of the same size and shape and arranged at the equatorial plate with the angle of the V turned in towards the center of the spindle, a characteristic arrangement of the chromosomes at this period. An equatorial section of a spindle in the same stage as Fig. 9, is seen in Fig. 10, where all twelve chromosomes are present. In this egg there are also found near the spindle a number of nucleoli which are in the process of disintegration.

Usually the first indication of any division of the chromosomes is seen at the stage of Fig. 11 when the polar arms have entirely disappeared and the chromosomes are broad V-shaped structures. At this time the ends of the V's often show a deep indentation (Fig. 11) indicating the longitudinal splitting of the chromosomes. Occasionally I have found the first division coming in at an earlier period before the entire disappearance of the polar arms. Such a division is seen in the chromosome at the left in Fig. 7. In all such cases the splitting is confined entirely to the lateral wings and never extends into the polar arms.

In the egg from which Fig. 12 was drawn, there are twenty-four V-shaped chromosomes which are similar to the twelve chromosomes in Fig. 10 in every respect except that they are much narrower. They have been produced, I believe, by a longitudinal division of the broad V-shaped chromosomes found at an earlier period. In some of the chromosomes shown in Fig. 12, the division for the second polar mitosis is seen. This second division of the chromosomes is not visible, at this stage, except in equatorial sections of the spindle. In longitudinal sections of the spindle the chromosomes always appear to be arranged in tetrad groups, one of which may be seen in Fig. 12. Such a group is, in reality, a pair of V-shaped chromosomes with the angle of each V turned in towards the center of the spindle, the four ends of a pair of chromosomes projecting from the spindle give the appearance of a typical tetrad. The maturation divisions of the chromosomes are represented diagrammatically by text-figures 1–5.

In my previous paper, three sections from one egg (Figs. 25, 26, 27) were given in which the fully formed spindle lay some distance below the surface of the egg and the chromosomes were in the form of closed rings which were split longitudinally. This egg was undoubtedly abnormal and led to the wrong inference that these chromatin rings were identical with those found in the germinal vesicle just previous to its disintegration. If the split V-shaped chromosomes of Fig. 11 were to be spread out in the form of a ring and the second maturation division to take place before the halves of the ring separated, then exactly the same effect would be produced as previously illustrated in Figs. 25–27. I can only interpret the ring-shaped chromosomes in this abnormal egg — the one abnormality I have found in many hundreds of eggs sectioned — as due to a delay in the separation of the parts after the two divisions of the chromosomes had taken place.



Diagrams of the maturation divisions of the chromosomes in the egg of Bufo lentiginosus.

According to Carnoy and Lebrun (2, 3) who have published a series of memoirs dealing with the development of the germinal vesicle and the formation of the polar bodies in the eggs of various Batrachia, the chromatin filaments in the egg of Salamander, *Alytes*, *Triton*, *Bufo* and *Rana* arise from repeated resolutions of the nucleoli in the germinal vesicle. As my own work on *Bufo* began with the fully formed egg taken from the animal just before the beginning of the hibernation period, I have not yet seen this resolution of the nucleoli into chromatin threads. In all the eggs that I have examined in which the germinal vesicle was intact, the chromatin was always in the form of distinct chromosomes. These chromosomes had no connection whatever with the large round nucleoli which, with the combination stain used, always stain a deep blue while the chromatin invariably takes the carmine. I have frequently noticed, however, that many of the chromosomes end in small granules which take the same stain as the chromatin and that there are a number of similar granules scattered throughout the·nucleus. Recent work on various forms has shown that unquestionably the term nucleolus has been applied to many different kinds of structures in the germinal vesicle. As a general term used to cover any definite structures in the germinal vesicle other than chromosomes, linin and karyoplasm, it may, perhaps, be fitly applied both to the large rounded structures (which I consider the only true nucleoli in the germinal vesicle) and to the smaller granules which stain like chromatin and which I believe to be chromatin that is not used for the chromosomes. The structures which, in my opinion, are the true nucleoli have nothing to do with the formation of the chromosomes for the first polar spindle, as they are never connected with the chromosomes in any way and can be traced step by step until they are absorbed by the cytoplasm of the egg after the spindle is completely formed.

Many of Carnoy and Lebrun's illustrations of the formation of the first polar spindle in the egg of *Bufo vulgaris* are strikingly like my own, but we differ somewhat in our interpretation of them. According to their view, when the germinal vesicle in the egg of *Bufo vulgaris* migrates towards the upper pole, and before the nuclear membrane disappears, the paired chromatin filaments (which are exactly like those I find in *Bufo lentiginosus* during the same period) break up into small granules which cannot be distinguished from the granules of karyoplasm. *All the nucleoli suffer the same fate as the chromosomes excepting about ten which remain to form the chromosomes of the first polar spindle.* The karyoplasm meanwhile, forms a pronounced radiation from the "plage fusoriale" at the lower pole of the germinal vesicle. "Les nucléoles prédestinés montent le long des filaments" and are carried to the "plage fusoriale" where they either become vacuolated in the center and form a ring, or else they fuse into one large mass and later regain their individuality. When the spindle is first formed, the chromosomes are very irregular in shape and there are distinct asters at the spindle poles which never contain a centrosome.

In the egg of *Bufo lentiginosus*, I have traced the chromosomes of the germinal vesicle up to the stage where the ends of a pair of chromosomes unite to form a closed ring. After this time, although I have had an abundance of material and have searched very carefully through every section of the germinal vesicle in a large number of eggs, I have been unable to find any trace of the chromatin. There is, I believe, no doubt but that the chromatin rings break up into minute granules which may, possibly, be carried by the karyoplasmic radiation to the lower pole of the germinal vesicle where they later form the chromosomes of the first polar spindle. I have never seen anything in this egg, however, that would indicate that some of the *nucleoli* are destined to form the chromosomes of the first polar spindle. A large number of nucleoli are always present throughout the early stages of maturation and they all appear to be undergoing the same processes of disintegration. Carnoy and Lebrun might consider the large irregular masses shown in Fig. 2 to be nucleoli in the general sense in which they seem to use the word, but these masses have been formed by the fusion of smaller chromatin granules (Fig. 1) and are in no way connected with the true nucleoli of the germinal vesicle.

Carnoy and Lebrun have followed the details of the formation of the first polar spindle and the later changes of the chromosomes much more carefully in the egg of *Triton* than in any of the other amphibian eggs they have studied. Their account of this form agrees substantially with that of *Bufo vulgaris* as regards the breaking down of the germinal vesicle, with the important exception that in *Triton*, *all* of the nucleoli are absorbed by the cytoplasm, none of them are reserved, as in *Bufo Vulgaris*, to form the chromosomes of the first polar spindle. The chromatin threads which were resolved from the nucleoli at an earlier period, break up into very small granules when the membrane of the germinal vesicle disappears. The twelve chromosomes which later arise *from a coalescence of the chromatin granules* are at first very irregular in shape and they are scattered all along the spindle fibers; subsequently they undergo a double longitudinal division in preparation for the giving off of the polar bodies. Any chromatin not used for the chromosomes is absorbed by the cytoplasm.

Although there are always twelve chromosomes on the first polar spindle in the egg of *Triton*, Carnoy and Lebrun find only 8–10 chromosomes in the equatorial plate of the first polar spindle in the egg of *Bufo vulgaris*, and but 4–5 chromosomes at each pole just previous to the giving off of the first polar body. The failure of these investigators to find the definite number of chromosomes that must be present unless the egg of *Bufo vulgaris* is a marked exception to the rule that the number of chromosomes is constant for a given species, may possibly be accounted for on the supposition that some of the chromosomes were lost when the eggs were sectioned or that the sections of the egg were made so thick that some of the chromosomes were not visible.

In a more recent paper, Lebrun (13) gives the results of a reexamination of the maturation processes in the egg of *Triton*. He states that the double longitudinal division of the chromosomes does not take place in the complicated manner previously described by Carnoy and Lebrun, but according to the scheme represented by my text-figures 1–5. The late maturation changes in the egg of *Triton* are, therefore, strikingly similar to those I have found taking place in the egg of *Bufo lentiginosus*. Lebrun still believes that in the eggs of *Rana temporaria* and of *Bufo vulgaris* a certain number of the nucleoli are reserved to form the chromosomes of the first maturation spindle. A reëxamination of the maturation stages in the eggs of these amphibians would probably show that in these forms also the chromosomes are derived from fused masses of chromatin granules and that they have no connection whatever with the true nucleoli.

I have examined a large number of the eggs of *Bufo* at the stages of Figs. 3–4 and I can see no reason for believing with Carnoy and Lebrun that a division of the chromosomes takes place at this time. During this period the chromosomes are exceedingly varied in size and shape. If the chromosome is oblong, it may have either its long or its short axis parallel with the longitudinal axis of the spindle; if the chromosome is pyramidal in shape, either the base or the apex of the pyramid may rest on the spindle fibers. I regard all of the changes in the shape of the chromosomes up to the stage of Fig. 7 as due solely to a rearrangement of the chromatin material preparatory to the later divisions. The first indication of any division of the chromosomes is the longitudinal splitting of the lateral wings which in some few cases can be found before the disappearance of the polar arms (Fig. 7). The apparent separation of the lateral wings at *X*, Fig. 11, I consider to be due to the fact that the angle of the V-shaped chromosome was cut off in sectioning. It very frequently happens that portions of one or of several chromosomes on a spindle are removed in this way. Sometimes, as in Fig. 4, the median knob of a chromosome is lacking; sometimes, the lateral wings have been removed (Figs. 6, 7). In rare instances the cut off portion of the chromosome will be found in the next section of the egg; but as the chromosomes are quite small a careful examination of the following sections often fails to disclose the missing part.

As found to be the case in many eggs besides that of *Bufo*, for example in *Cerebratulus* (Coe), *Polychærus caudatus* (Gardiner), *Thalassema* and *Zirphæa* (Griffin), and *Triton* (Carnoy and Lebrun), all the chromatin of the germinal vesicle does not go to form the chromosomes of the first polar spindle, some of it is thrown out into the cytoplasm where it degenerates and sooner or later completely disappears. Even in the segmentation stages of the egg of *Ascaris*, Boveri (1) found that some of the chromatin is thrown out of the nucleus and absorbed by the cytoplasm. In all these cases there is obviously a mass reduction of the chromatin in preparation for the succeeding division of the cell. It may be, as suggested by Gardiner, that "there are two kinds of chromatin stuff, the one insoluble and bearing the heredity which is to be transmitted to the daughter cells, and the other food for the cytoplasm." This theory would explain the facts as we now know them, but it cannot be proved until some stain can be found to differentiate the two sorts from each other.

Carnoy and Lebrun find a double division of the chromosomes in the egg of *Triton*, and they state that there is no reason why a longitudinal division of the chromosomes should not be a reduction division in the Weismann sense, in that it may separate the chromosome into two parts each containing different kinds of granules: it is certainly true if we admit a difference in the properties of the elementary granules. As all of the chromatin granules do not go into the chromosomes of the first polar spindle, there is a process of selection in the formation of the chromosomes and their subsequent division would be a permanent source of variation for the descendents.

The chromosomes of the first polar spindle in the egg of *Bufo lentiginosus* are at first exceedingly varied in shape; they may be round, triangular, or oblong. At this time it is obvious that they have no definite longitudinal axis. At the stage of Fig. 5 the chromosomes have elongated and lie parallel with the longitudinal axis of the spindle. When the wings have formed, there is a stage when the arms of the chromosomes are all approximately of the same length (Fig. 6). Is there a definite longitudinal axis at this time? If the part of the chromosome resting upon the spindle fibers is considered to be the longitudinal axis, then later this same axis is not only shorter than the transverse axis, but it practically disappears at the stage of Fig. 9. If shown Fig. 9 without the preceding figures, no one, I am sure would call the thickness of the chromosome at the angle of the V the longitudinal axis of the chromosome, and the division indicated in Fig. 11 would unhesitatingly be called a longitudinal division. If one arbitrarily states that the polar arms of the chromosomes in Fig. 5 form the true longitudinal axis, not only in this particular stage, but until division is completed, then the splitting seen in Fig. 11 is a transverse division, as is also the second division which takes place in the same direction. On the other hand, if the longer axis of the chromosome at the time when division occurs is considered to be the true longitudinal axis, then there is a double longitudinal division of the chromosomes and the egg of *Bufo* is thus brought into line with other amphibian eggs that have been studied. It would seem, as suggested by Sebaschnikoff (14), that the distinction between transverse and longitudinal divisions of the chromosomes is not as important as many investigators have claimed: the division of the chromatin substance would appear to be the important thing, the manner of its achievement quite secondary, as Hertwig (9) has maintained.

There is, however, the following possibility to be considered. When the germinal vesicle breaks down, all of the chromosomes are arranged in pairs, in some cases the ends of a pair of chromosomes have united to form a closed ring. Very soon after this stage the chromosomes break up into granules and all traces of the chromatin substance is lost until innumerable chromatin granules appear in connection with the first polar spindle. It is conceivable that all of the chromatin granules belonging to a pair of chromosomes have remained united during this period of the apparent disintegration of the chromosomes, although I have not been able as yet to demonstrate such a union. If such is the case, then the chromosomes of the first polar spindle are bivalent structures, each being composed of the two chromosomes that had become paired at an earlier period of development. On this assumption it is probable that the knob-like thickening in the middle of the chromosomes, shown in Figs. 4 and 5, is caused by the fusion of the ends of the two chromosomes. In text-figure 1, *ABC* and *ACD* would represent the two chromosomes united at *AC*. The subsequent changes in the shape of the chromosomes serve merely to again elongate the original chromosomes (Text-fig. 3) which are finally separated by the division through *AC*. The first maturation division, therefore, is a reduction division and the second division only is a longitudinal one. It certainly cannot be mere chance that at the time of the breaking down of the germinal vesicle, the chromosomes should invariably become arranged in pairs. In light of the most recent investigations on spermatogenesis and ovogenesis it would seem as if the above explanation must be the true one for the maturation divisions in the egg of *Bufo*, although at present I am not able to prove it. I hope that the work I am doing on the spermatogenesis of this amphibian will throw some light on the maturation divisions in the egg.

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EXPLANATION OF PLATE.

All figures were drawn with the aid of a camera lucida under a Zeiss Apoc. 2 mm., Oc. 4.

1. An early stage in the formation of the first polar spindle before the chromatin granules have fused into large masses.

2. A stage about one-half hour later than Fig. 1. The spindle has become barrel-shaped and the chromatin granules are fusing into large masses to form the chromosomes.

3. Twelve irregularly shaped chromosomes have differentiated from the chromatin masses and lie scattered along the spindle.

4. Spindle parallel to the surface of the egg. The chromosomes have elongated and many of them show a median knob. *C.M.*, chromatin mass outside the spindle.

5. About the same stage as Fig. 4. Chromosomes of very different sizes are found on the spindle.

6. Typical "oiselet" stage.

7. Chromosomes in various stages of development on the same spindle. In some of the chromosomes the splitting for the first maturation division can be seen while the polar arms are still present.

8. Section showing the growth of the lateral arms of the chromosomes at the expense of the polar arms.

9. The V-shaped chromosomes after the disappearance of the polar arms.

10. An equatorial section of a spindle at the stage of Fig. 9. All twelve chromosomes are present.

11. Equatorial section. The notched ends of some of the chromosomes indicate the direction of the first maturation division.

12. Equatorial section. The first maturation division is completed and the second maturation division is indicated in some cases.

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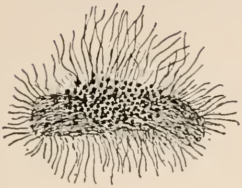

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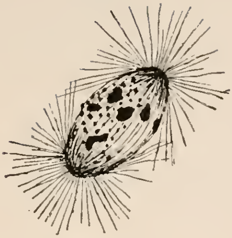

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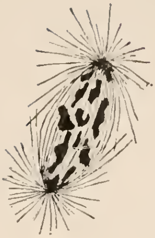

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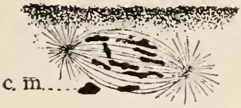

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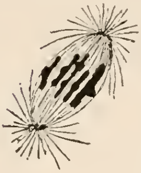

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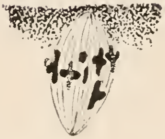

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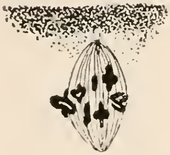

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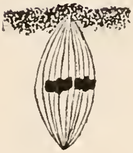

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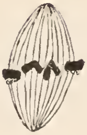

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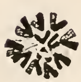

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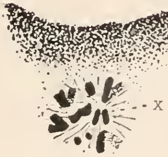

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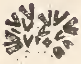

THE BREATHING AND FEEDING MECHANISM OF THE LAMPREYS. II.

(*Concluded.*)

JEAN DAWSON.

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II. Food and Mechanism of Feeding.

A. *Mode of Attachment.* — If a living *Lampetra wilderi* or an *Ichthyomyzon concolor* (Kirtland) be placed in a glass dish its mode of attachment to the sides of the dish may be observed. The animal, when swimming freely, brings the sides of the oral funnel together so that it forms a vertical wedge-shaped cutwater which offers less resistance to the water than the open funnel. When it is about to attach itself, the closed funnel quickly opens against the glass and, with a single rapid backward and forward stroke of the tongue, the animal becomes firmly attached. The tongue in moving forward resumes its normal position in contact with the surface of attachment and is not seen to move again during the time of attachment, unless one attempts to pull the animal away in which case the tongue is seen to move as in the case of the first attachment. When the lamprey becomes accustomed to being handled it will seize one's finger and a strong suction may be felt resulting from the action of the tongue in the process of attachment.

If the oral funnel of a recently dead lamprey be pressed against the dish or finger, it becomes attached. If now one pulls the body backward in a direction at right angles to the surface of the attachment, it is found to be firmly attached. This attachment is so firm that one may lift from the water a dead lamprey thus adhering to the finger. If, instead of pulling the lamprey backward or away from the surface, one pushes it in any direction parallel to the plane of attachment, the oral funnel glides easily over the surface of attachment. When the lamprey is attached to a moving fish, the weight of the body as it drags through the water exerts a backward pull on the lamprey in spite of which the animal is able to maintain its hold, though at the same time it is free to glide in any direction over its host.

Since the dead lamprey remains attached, it is clear that the maintenance of the vacuum which effects the attachment is not through muscular action, but through mechanical means. Thus it is probable that the living lamprey may at times remain attached to the host without the expenditure of any muscular energy.

Is the vacuum by which the animal maintains its hold formed in the mouth cavity alone or in both mouth and pharynx? To determine this, the following experiments were tried on *Lampetra wilderi.* (1) A capillary glass tube was fitted over a cambric needle so as to form a small trocar. This was inserted into the nostril of a lamprey and so directed that the needle pierced the dorsal wall of the pharyngeal cavity. The needle was then withdrawn leaving the tube in the opening. The tube was occasionally cleaned by re-inserting the needle. The animal in which a communication was thus established between the pharyngeal cavity and the exterior was able to attach itself as firmly as before, thus showing that the vacuum is formed not in the pharynx but farther forward, *i. e.*, in the mouth cavity.

(2) A glass tube was inserted into the mouth cavity of a second lamprey between the lobes of the tongue. This tube was just long

enough to reach from the mouth opening to a point about 1 cm. back of the semiannularis muscle and establish a connection between the mouth cavity and that of the pharynx. At first the animal made fruitless attempts to attach itself and finally did so as firmly as ever. Upon examination, the animal was found to have swallowed the tube. A tube of the same length, but having a hook at one end, was now inserted into the mouth as before. The hooked end lay between the tongue lobes but did not interfere with the oral funnel in any way. It was found that the lamprey was neither able to swallow the tube nor to attach itself but lay on its side. Finally it was able to attach itself very feebly, but in order to do so the tongue worked back and forward continually, thus creating a slight vacuum.

(3) A guarded bristle was passed through the second external gill opening of a living lamprey into the water tube and on through the mouth so that the enlarged tip of the bristle lay between the tongue lobes, but did not interfere with the action of the funnel. The bristle thus prevented the complete closure of the velar valves and also the close approximation of the lateral tongue lobes. The animal was unable to attach itself except feebly by the aid of the continuous movement of the tongue already described. The bristle was drawn back of the semiannularis muscle so that it could no longer interfere with the action of the tongue, but was left where it would interfere with the action of the velar valves. The animal immediately regained the power to attach itself as firmly as before, thus showing that the velar valves do not aid in forming the vacuum in the mouth cavity.

From these experiments it follows that the partial vacuum which effects the attachment of the lamprey exists in the mouth and oral funnel only. The movement of the tongue at the moment when the animal attaches itself shows that the vacuum is produced at least in part by the pumping action of the tongue. The fact that after the animal has attached itself, the tongue projects forward, shows that the communication between the mouth cavity and the pharynx is not kept closed by the tongue, but probably by the semiannularis muscle, thus leaving the tongue free for other purposes.

In the case of the dead lamprey, the pumping action of the tongue must be replaced by the pressure exerted by the fingers against the oral funnel in the act of attaching the animal, the tongue then remains in the back part of the mouth cavity and thus functions in place of the semiannularis to close the communication between the mouth cavity and the pharynx.

B. *Mode of Feeding of Attached Lampreys.* — This has never been observed, nor is it likely to be, so that our knowledge of the process of feeding is an inference from the structures involved. The moment the animal attaches itself the oral funnel is opened quickly and is pressed flat against the supporting surface. The process may be watched when the lamprey attaches itself to the sides of a glass dish. The funnel may be so much flattened that the teeth on the tongue press against the glass.

In this position the teeth of the oral funnel and tongue constitute a very efficient rasping apparatus. Fürbringer (1875) has shown how the teeth of the funnel are moved in and out on radial lines by the action of the inner layer of the annularis muscle. The arrangement of teeth as already pointed out is such that in their movement along radial lines they necessarily lacerate every part of the surface covered by the funnel. It will be shown below that the tissues of the host are thus reduced to a fine pulp, which is then swallowed.

We may infer that this pulp finds its way into the pharyngeal cavity by the pumping action of the tongue. The considerable size of the pharnygeal cavity (Fig. 4) and the distensibility of its walls enable it to accommodate a large amount of food. By the contraction of the muscular wall, particularly the pharyngeus muscle and the basilaris muscles, the food is forced back into the œsophagus, while the posterior pharyngeus muscle relaxes to permit its passage.

C. *Character of Food.* — As regards the character of the food, there is much difference of opinion. Günther (1853) says: "Die Nahrung des Neunauges besteht ausser Würmern, Insekten, etc. noch in Fischen, sowohl kleinen, als grössern, an welche sie sich wie an Steine festsaugen." Günther does not indicate that he makes this statement from the examination of stomach contents of the lamprey, so that it is possibly merely a current belief.

Abbott (1875, p. 827), speaking of *Petromyzon marinus* says: "This fish which is found occasionally hibernating in the soft mud at the mouths of some of the inflowing creeks appears to come from the bay or ocean (at any rate from the lower portion of the river) in immense numbers early in March and remains about the rocks at the head of tide water for some time as though waiting for the coming shad or herring. With the shad they pass up the river beyond tide-water, and in the rapid rocky portions of the river having deposited their own ova, they wander over the breeding grounds of other fishes and devour every egg they can find. I have found lampreys in Crosswicks Creek in the month of May gathering up the eggs from sun fish nests." But here again the observation is not supported by an examination of stomach contents.

The only other observation known to me is that of Gage (1893) on *P. marinus unicolor.* "Of all the specimens obtained out of the breeding season either the digesting part of the alimentary canal was empty or it contained blood. No partly digested worms or insects or small fish or parts of fish flesh were found, although diligent search was made; consequently it is believed that the lake lamprey is wholly parasitic during its adult life and lives on blood sucked from other fishes." Again (p. 438) he speaks as follows of the intestine at the breeding season: "The atrophy takes place within two weeks, and begins at the terminal extremity, and extends gradually cephalad until the whole canal appears like a thread. As no food is taken during the spawning season there is no necessity for digestion, and in the female there is no room for the intestine when the ova are completely matured."

As it seemed incredible that the very elaborate rasping apparatus of this lamprey could have no other use than to enable the animal to produce a raw surface from which to suck blood, a thorough investigation of all the material of *P. marinus* at hand was made. But in all cases the intestines were found to be empty and atrophied. It was then learned that all the available lampreys had been caught in the breeding season. Through the kindness of Professors Gage and Wilder, however, I obtained several specimens of *Petromyzon marinus unicolor* taken in the breeding season and one specimen caught in December. The lampreys caught in the breeding season showed the same atrophied condition of the intestine that had been found in our own material, but the intestine of the one that was caught out of the breeding season was found to be gorged with food. The intestine measured 4 cm. in circumference while the intestine of a specimen of the same size caught in the breeding season measured but 6 mm. The œsophagus was entirely empty. Among the contents of the intestine solid particles could easily be seen with the unaided eye, while a microscopic investigation proved most of these to be muscular tissue. The more liquid parts were past recognition. There were recognizable bits of striated muscle about 12 mm. long, a gill and a rib of a small teleost fish. The gill was 1 cm. long and bore filaments 5 mm. in length; the rib was 2 cm. long. It is impossible to tell whether the lamprey came by this small fish directly or from the intestine of a larger fish which served as its prey.

In any case it appears that the attached *P. marinus unicolor* may feed not only on blood but on more solid tissue. The very extensive injuries produced by this species and figured by Surface (1893) are in accord with this conclusion. Presumably *P. marinus* and *Ichthyomyzon* have similar habits. According to Gage (1893) the adult *Lampetra wilderi* takes no food.

III. Mechanism of Respiration.

A. *When the Lamprey is Attached.* — Respiration may readily be studied when the lamprey is attached, and has been described by Bert (1867), Gage (1893) and Meyer (1835). The respiratory currents may be easily seen in *L. wilderi* and in *Ichthyomyzon concolor* by means of particles suspended in the water. There is a rapidly alternating contraction and expansion of the branchial region. At each contraction a current of water leaves all the external branchiopores simultaneously and passes outward and backward at an angle of 45° with the long axis of the body. At the same time a current issues from the nostril. With the expansion of the branchial region the water is seen entering the external branchiopores in lines converging from all directions to each aperture and at the same time a similar current enters the nasal opening.

Many factors vary the rate of the respiratory movements, such as the vitality of the individual animal, temperature and the oxygen content of the water.

The movement when the animal is attached is regular unless foreign particles get into the gills, in which case spasmodic contractions of the gills take place in order to expel the irritating particles. These contractions are essentially the same as in regular respiration except that they are longer continued and stronger, so that the external branchiopores are brought closer to the water tube. If many solid particles are suspended in the water the rate of breathing becomes slower and may even stop for a full minute, but if the animal is removed to clean water breathing again becomes normal.

During both expiration and inspiration the external branchiopores are wide open and look like so many gaping, round mouths. They do not change their form in either inspiration or expiration. The ectal valves are not taut; with the aid of a lens the ectal valves may be seen flapping idly back and forth in the opening (Fig. 10, *a*). With each inspiration the ental valves are seen to be swept back into the gill pouch much as swinging doors might be. At each expiration the out-flowing water sweeps the ental valves forward and out through the external gill opening past the loose border of the ectal valve (Fig. 10, *c*). It is thus clear that in the normal respiration of the attached lamprey the valves of the external branchiopores have no office other than to perhaps aid in directing the out-flowing water backward.

The movements of respiration and the working of the ental and ectal valves could be seen somewhat more plainly in *Ichthyomyzon concolor* than in *L. wilderi.* Besides the regular respiratory movements recorded for *L. wilderi* a slight backward and forward motion of the branchial basket was observed.

From the arrangement of the muscles of the gill sacs and gill pouches it may be inferred that the expulsion of the water is brought about by contraction in all directions of the lumen of the gill sac, while at the same time the long axis of the sac is more shortened than any of its other dimensions. Thus the capacity of the gill sac is reduced and a part of the water is expelled. The lateral end of the muscular pouch carries inward with it the elastic branchial basket to which it is attached. When the muscles relax, the elasticity of the branchial basket serves to elongate the gill sacs and thus to fill them again with water. The action may be likened to that of the mammalian lung in which the lung is filled by muscular action and emptied chiefly by the elasticity of the thoracic walls and lungs. The gill sacs of the lamprey are, on the other hand, filled by the elasticity of the branchial basket and emptied by muscular action. Not all the air in the lungs is changed by a single respiration and not all the water in the gill sacs can be changed at a single respiratory movement. The expulsion of the water at the angle of 45° does not retard but rather aids the progressive movement of the fish, while it permits the external branchiopores to have such a form and point in such a direction that they offer the minimum resistance to the movement of the animal through the water.

Gage's (1893) statement that the expired water leaves the external branchiopore at a very oblique angle with the long axis of the body while the inspired water enters at an angle of 90° suggests the possibility that during expiration the major axis of the gill sac forms a very oblique angle with the long axis of the body but that during inspiration this angle becomes a right angle. The following experiment was tried to find out whether there was any change in the obliquity of the gills during respiration: A bristle with as large a tip as would enter the external branchiopore was passed through the gill sac into the water tube so that it occupied the major axis of the gill sac. The end of the bristle was left projecting from the external gill opening and formed an angle of 45° with the long axis of the body. This angle remained constant during both inspiration and expiration.

Gage (1893, p. 469) believes this arrangement by which water leaves the gills at a very oblique angle while the inspired water enters at an angle of 90° is a contrivance to prevent the repeated respiration of the same water. The water does indeed leave the gill at a rather oblique angle (45°), but if the observations here made on the current entering the gill openings be correct, the water flows in from all directions much as it enters the mouth of an empty submerged bottle; moreover it is difficult to see the need of a contrivance to prevent the repeated inspiration of the same water. The expired water is thrown out with great force and to a considerable distance like a stream from a hose. It is thoroughly mixed with the adjacent water which by this means as well as by the movements of inspiration is kept constantly agitated. When we remember the rapidity with which gases diffuse through water thus agitated (Hoppe Seyler, 1896) there can be little doubt that the water inspired by a *Petromyzon* is of practically the same gaseous content as the adjacent water and is unaffected by the expired water. Probably a special contrivance for preventing the repeated inspiration of the same water is little more needed in *Petromyzon* than a similar contrivance for preventing the repeated inspiration of the same air in a mammal.

The current seen issuing from the nostril at each expiration is caused by the compression of the nasal cœcum. The caudal end of this cœcum is bounded above by the cephalic end of the notochord and below by the first two gill pouches (Fig. 1, *f*). As the gill sacs shorten with each expiration the cœcum is pressed against the notochord and water is forced from it. With the lengthening of the gill sac the walls of the cœcum are again separated and water is drawn into it. This current seems to have no other purpose than to bring the water in contact with the olfactory epithelium.

B. *Respiration when the Animal is not Attached.* — The acts of feeding and breathing have now been considered in the normal attached animal, and no use has been found for the velar valves, the velar jaws, the water tube or the valves of the external branchiopore.

When a gill sac expands there is created a negative pressure within it. To relieve this, water must enter the sac either through the internal branchiopore or through the external branchiopore or through both. When the animal is attached water enters necessarily through the external branchiopore alone. When the animal is not attached the mouth is open and water must enter through the external branchiopore, the valves of which are so constructed that they can offer no resistance to an inflowing current, but water may also conceivably enter the mouth, pass the velar valves and enter the gill sacs by way of the water tube and internal branchiopores. Thus both when the animal is attached and when it is not attached, water must enter the external branchiopores during inspiration. When the animal is not attached the water thus inspired through the external branchiopore may possibly be mixed in the gill sac with water inspired through the mouth. Indeed the inspiration of water through the mouth may be one means by which the unattached lamprey secures food. It is generally believed among writers (Mayer, 1835; Gage, 1893; Couvreur, 1897) that water may be inspired through the mouth of the lamprey when unattached. It is possible that breeding lampreys behave differently in this respect from those that are not breeding, but none of these authors state the time of the year in which their observations were made.

In order to ascertain whether water is normally inspired through the mouth, the following experiment was tried. A breeding *Lampetra wilderi* was placed in a wire basket which was in turn immersed in a dish of water. The meshes of the basket were small enough to keep the animal from getting out, but afforded no surface to which the animal could attach itself when it came to rest. A carmine mixture was placed near the mouth of the animal when it was thus quiet and unattached, but no current of water could be seen passing into the mouth. Many trials were made, but with the same result. This led me to the conclusion that in the adult lamprey, of this species at least, no current of water is taken into the mouth, but this is of course not true of the larval form.

On November 29, 1904, two living specimens of *Ichthyomyzon concolor* (Kirt), were obtained from the Detroit river and placed in a large aquarium. When one of these was placed on its back without unduly exciting it, the animal immediately became quiet and after a short time, while it remained in this position, could be handled as though it were dead. As soon as it assumed its normal position, however, the animal became active again. The phenomenon seemed to be of a hypnotic nature and obviated the necessity of giving chlorotone to quiet the animal.

While the animal lay on its back a mixture of carmine and water was poured into the upturned oral funnel. Now and again a red current of water could be seen passing into the mouth and out through the external branchiopores. After a few inhalations, the current would be reversed and a stream of water would be sent out of the mouth with some violence as though the carmine in the water was found to be irritating. The current of water passing into the mouth, it will be remembered, was not seen in the *L. wilderi*.

The two specimens were kept alive for five weeks in the laboratory when they died after being attacked by fungus. During their life in the aquarium, they were not observed to have taken any food, although large pieces of raw beef were suspended in the aquarium.

The only advantage to the lamprey of an inspired current through the mouth would lie in the fact that by means of such a current food might be caused to enter the mouth when the animal was free. The experiment on the inspiration of water through the mouth in *L. wilderi* above recorded was necessarily performed during the breeding season when the brook lamprey takes no food. It is therefore inconclusive. The similar experiment on *I. concolor* performed out of breeding season would tend to support the statement of Günther (1853) and Abbott (1875) that the lampreys feed when free as such feeding could hardly be accomplished without the inspired current through the mouth.

C. *Detachment and Regurgitation.*—The valves of the external branchiopores are clearly useless in aiding the animal to draw water into the mouth. There remains but one use for these structures, namely, to render possible an expired current through the mouth.

When the lamprey is firmly attached there is a partial vacuum in the mouth cavity and the oral hood. It would require considerable force for the animal to tear itself free when thus attached, but if by closing the external branchiopore and opening the velar valves and mouth it can force water into the oral funnel from the gill sacs, the vacuum will be at once destroyed and the animal may free itself without great muscular exertion. There is a further possible advantage to the animal in being able to expel a current of water from the gill sacs through the mouth. Such a current would enable it to cleanse the pharynx and mouth of indigestible and bulky food particles, nor does there appear to be any other means of accomplishing this.

In order to learn how the animal detaches itself the following observations were made : The ectal and ental valves of the external branchiopore were watched very closely with a lens to determine their action, if any, at the moment when the animal detached itself. This proved to be no easy task since the valves were found to act so quickly that one could see but a flash of white in the dark gill opening and the animal was gone before one could see what had happened. This difficulty was greatly lessened by the use of a small amount of chloretone in the water. This increased the rate of breathing at first but then gradually lowered it so that one could more easily tell what was taking place.

The cephalic and caudal sides of the gill opening were seen to be approximated so that instead of remaining circular, as during ordinary respiration the opening became elliptical with its longest axis dorso-ventral. Thus the ectal valve was stretched taut. The ental valves could be seen to come together so as to close this gill opening. Almost the instant the valves closed, the animal detached itself.

It thus appears that the valves of the external branchiopore, act in the manner indicated by their structure, to close the branchiopore at the moment when the animal detaches itself. Experiments were now tried to see if the water was sent to the mouth cavity to destroy the vacuum as it seemed from a knowledge of the workings of the valves and their action at the moment of detachment of the animal that it might be.

Experiment 1.—The head of a lamprey while attached to the side of a glass dish was gently pushed up out of the water without detaching the animal. Care was taken to thoroughly dry the the glass around the oral funnel. When the animal detached itself, water was seen to run down the side of the dish from the mouth.

If the lamprey's head and several gills were thus lifted above the water, air bubbles and water are expelled from the mouth upon the animals detaching itself. The air bubbles must have been taken in through the gill openings which were above the water and must have passed forward through the water tube and pharynx into the oral funnel.

Experiment 2.—A thin mixture of carmine and water was introduced into a gill of a *L. wilderi* attached to the side of a glass dish, by placing the end of a pipette over the external branchiopore and gently but steadily pressing the bulb. The animal expelled the carmine from the gill opening with violence. When a thicker mixture was used, the animal made a great effort to expel it, but when the carmine was still steadily poured into the gill, the animal detached itself and a stream of red was seen to issue from the mouth. When carmine was seen to issue from the mouth, no carmine issued from the gills, thus showing that the valves were acting to close the external branchiopore and to cause the water to take a forward course so as to make detachment easier for the lamprey. These experiments seem to leave no doubt that detachment is effected in *Lampetra wilderi* by a current of water forced from the gill sacs into the mouth cavity and that this is rendered possible by the closure of the external branchiopore by its valves.

As far as I have been able to find, the valves of the external branchiopore are mentioned by but two writers.

Mayer (1835) speaks of two flaps at the external branchiopore and describes them as being swept out and in through the branchiopore by the respiratory current. He assigns no function to them except that of forming a tube for the outflowing water.

Gage (1893) says: "In the case of the lamprey one might think at first that no valves were necessary in respiration, for if the branchial pouches are open to the surrounding medium through the branchiopores any enlargement of the branchial spaces would cause the water to enter, and conversely, any constriction would empty the branchial sacs. This view is correct but this mode of simply drawing water into a sac and expelling it has not apparently answered the requirements of the lamprey, and there is present the thin valve (the ectal valve) which covers the entire branchiopore in the larva, and in addition a double valve (ental valve), which is formed by the growth and modification of the middle gill lamella of the caudal half of the branchial sac." Concerning the function of the valves he says: "In inspiration the two parts of the inner or ental valve turn away from each other and are pressed toward the cephalic wall of the branchiopore across the channel at the edge of the branchial sac, and the ectal or transverse valve folds over the ental one. By the expansion of the branchial apparatus, the entrance to the gill sac has been rendered more direct and the inflowing stream flows directly into the sac. In inspiration, the water flows through the branchial lamellæ, while around the edges, *i. e.*, at the dorsal and ventro-lateral edges of the gill sac there is formed a canal or gutter by the shortening of the gill lamellæ. The free ends of the lamellæ are also membraneous and curved and aid in making a very complete and smooth canal. The ental valves at the entrance to the branchiopore cross this canal and serve as a guide to the inspiratory stream, not allowing the water to get into the canal around the edges of the gill sac, but directing it into the gill sac itself. In expiration, however, with the change in obliquity and the constriction of the gill sac, the water passes between the branchial lamellæ into the canal and meeting the ental valve rotates the two folds of the valves toward each other and against the caudal wall of the branchiopore, thus removing the obstruction in the canal and really extending it by means of the arched valves. From this arrangement it is seen that two distinct objects are attained, the water not only bathes the gills, but passes between the lamellæ, it is then concentrated in a canal with smooth sides where the friction is at a minimum and in its exit from the branchial sac in expiration the valves prevent the used water from making a circle in the gills, and more important, they form a very oblique channel which directs the expiring stream caudad, thus insuring the animal against using the water over and over. In inspiration, on the other hand, from the direction of the opening, the water enters at nearly a right angle to the axis of the animal, and thus fresh or unrespired water is constantly supplied to the gills."

It has been shown above that these valves have another function than the one indicated by Gage. That they may also act to direct the current of water within the gill sac as Gage believes, does not seem to the writer necessarily to follow from the observations on record. The course that water may take within the continuous space of the gill sac, during the inspiration and expiration seems to be determinable only by direct observation or by experiment, the inflowing stream may indeed be directed toward the center of the gill sac by the ental valve, but when the gill sac begins to empty itself the ental valves respond to the slightest current, the branchiopore is unobstructed and all the water within the gill sac is equally free to pass out through it. It would seem that the water should then flow directly out through the branchiopore rather than that it should take the circuitous course between the gill lamellæ into the smooth channel along the dorsal and ventral borders of the gill sac and thence out.

D. *The Velar Jaws.* — Mayer (37) gives a figure in which the velar jaws are shown, but gives no description of them.

Stannius (1840) speaks of thread-like projections from the cartilage of the velar valves and may thus possibly refer to the velar jaws.

Vogt and Yung (1889) described the velar jaws as a straining apparatus. From the cartilages of the valves "extend five long thin forked points directed forward with their converging ends and thus a strainer is formed which opposes the entrance of bodies from the pharynx."

I have examined the velar jaws of many specimens of *P. marinus* but have never seen them with projections or otherwise than smooth. That the jaws would act as a strainer and serve to hinder the entrance of foreign bodies into the water tube is clear from their position. From the fact that they close when the velar valves close, it is clear that they may seize and hold foreign bodies which are being carried into the water tube. If the velar valves should then open to permit the forward current from the water tube to enter the pharynx, the velar jaws would be opened by the same muscular contraction, the foreign body would be released and swept forward out of the mouth.

Nevertheless pending the examination of stomach contents the question as to what extent, if at all, the lamprey takes food by means of a current of water entering the mouth must be regarded as still open. If food is thus taken, it is quite possible that as food particles are swept past the velar jaws into the water tube the jaws may seize the larger particles, permitting the smaller ones to pass on with the inspired water. The closure of the jaws would be accompanied by the closure of the velar valves and the stoppage of the inflowing stream of water. If the velar valves were then opened, the jaws would open and the food would be released and if at the same time the œsophagus should open by relaxation of the posterior pharyngeus muscle, the jaws lie so near the opening of the œsophagus that the food with a small quantity of water would be swept into the œsophagus. The more one considers the mechanism, the more does the conviction deepen that the lamprey is able to feed by means of a water current through the mouth and by the aid of the velar jaw.

Summary.

1. When *Lampetra wilderi* is swimming, the sides of the oral funnel are approximated so that the opening into it is reduced to a narrow vertical slit, fringed by the oral cirri. The compressed oral funnel then serves as a vertical wedge-shaped cut-water.

2. When *Lampetra wilderi* is about to attach itself to any surface, the oral funnel is spread and applied to the surface with a quick backward and forward movement of the tongue and the animal becomes attached.

3. If an opening is made between the pharyngeal cavity and the exterior in *Lampetra wilderi*, the animal is still able to attach itself in the same manner as though uninjured.

4. If a communication be established between the mouth cavity or cavity of the oral funnel and the pharyngeal cavity or the exterior, the animal is unable to attach itself.

5. Attachment in *Lampetra wilderi* is therefore effected by means of a partial vacuum created in the cavities of the oral funnel and mouth by piston-like action of the tongue.

6. A dead *Lampetra wilderi* becomes firmly attached when its oral funnel is pressed against a surface with the fingers, and remains thus attached.

7. It follows from six that an attached *Lampetra wilderi* does not necessarily exert muscular energy to maintain its hold.

8. The oral funnel of a dead or living *Lampetra wilderi* may be moved about freely and very easily over the surface to which it is attached so that the animal is enabled to glide over the surface of its host and so change its position with ease.

9. The oral funnel of a dead or living *Lampetra wilderi* may

be pulled at right angles from the surface to which it is attached only by the exertion of considerable force.

10. It follows from 9 that *Lampetra wilderi* attached in a current of water may retain its hold without necessarily exerting muscular energy.

11. In inspiration in the attached *Lampetra wilderi*, water enters each external branchiopore and the nasal tube, coming gently from all directions, just as it enters the mouth of an empty, submerged bottle. It does not enter merely at an angle of 90° to the long axis of the body as stated by Gage for *Petromyzon unicolor*.

12. In expiration water leaves each external branchiopore in a backwardly directed stream which forms an angle of 45° with the long axis of the body. At the same time a stream issues from the nasal sac.

13. No muscular mechanism has been found to account for the expansion of the gill sacs by means of which inspiration is effected and the inpiration is therefore attributable to the elasticity of the cartilaginous branchial basket.

14. During expiration the gill sacs are compressed and their long axis shortened by the action of the following muscles: *a*, the internal and external compressors of the gill sac; *b*, the superficial compressors of the gill pouch (a muscular pouch which encloses each gill sac and is separated from it only by a large lymph space).

15. Water is forced out of the nasal opening at each expiration by the compression of the nasal cœcum between the notochord and the adjacent first and second gill pouches.

16. Water is drawn into the nasal opening at each inspiration by the expansion of the nasal cœcum due to its attachment to the adjacent medial walls of the second and third gill pouches which are separated by the elongation of the axis of these pouches during inspiration.

17. During both inspiration and expiration the external branchiopore has approximately circular form and the valves guarding it (ectal and ental valves of Gage) are seen to flap idly in and out of the opening.

18. The ectal valves may, as claimed by Gage, help to direct the inflowing current of water to the central part of the gill sac,

but the writer has obtained no evidence that this is the case, nor does she see any reason to believe that during expiration there is a movement of the water within the gill sac in the definite manner indicated by Gage.

19. In the attached *Lampetra wilderi* and *Ichthyomyzon concolor* in normal respiration, the ectal valve of Gage is relaxed, that is, its free edge is not stretched and no other function is observable in it than that of aiding in directing the expired current of water.

20. At the moment when *Lampetra wilderi* or *Ichthyomyzon concolor* detaches itself the dorso-ventral axis of the external branchiopores is seen to elongate so that the free border of the ectal valve is stretched taut. The ental valve at the same time strikes against the inner surface of the ectal valve and is thus prevented from turning outward. The external branchiopore is thus closed.

21. The elongation of the dorso-ventral axis of the external branchiopore is due to the contraction of the muscles, the ectal and ental, which act upon the cartilaginous ring to which the valves are attached.

22. If the head of a *Lampetra wilderi* is above the surface of the water, at the moment of detachment, a few drops of water are seen to issue from the oral funnel. If the head of an attached lamprey is far enough above the surface of the water so that two or more branchiopores are exposed, air and water issue from the mouth when the animal detaches itself. If carmine laden water has been introduced into the gill sac of an attached animal the head of which is submerged, a red stream is seen to issue from the mouth at the moment of detachment.

23. It is concluded from 20, 21 and 22, that detachment is effected by a current of water directed forward from the gill sacs through the water tube and pharynx so as to destroy the vacuum in the mouth and oral funnel.

24. If a thin mixture of carmine in water be introduced into the gill sac of a free *Lampetra wilderi* or *I. concolor* by means of a pipette held opposite the external branchiopores, the fluid is expelled from the external branchiopores by contraction of the gill sacs somewhat more violent than those of ordinary respiration.

25. If a thick carmine mixture be introduced as under 24 it

frequently happens that a violent contraction of the branchial region follows, accompanied by a discharge of the carmine from the mouth. At the moment of this discharge the external branchiopores are seen to be closed as noted under 20.

26. If a stream of thick carmine mixture be directed gently into the oral funnel of a free *Lampetra wilderi* or *Ichthyomyzon concolor* so as to fill the funnel, it frequently happens that there is a violent contraction of the branchial sac, accompanied by a stream of water which issues from the mouth and expels the carmine solution. At the same time the external branchiopores are seen to be closed as noted in 20.

27. It is concluded from 25 and 26 that a current of water from the gill sacs forward through the water tube, pharynx and mouth cavities is the agent by means of which these lampreys habitually cleanse pharyngeal and mouth cavities and is the only means by which bodies too large to pass into the alimentary canal or through the branchiopores, may be removed.

28. The current of water directed forward through the mouth, by means of which these lampreys are able to detach themselves and to cleanse the mouth cavity and pharyngeal cavity affords the first adequate explanation of the function of the valves of the external branchiopore, of the water tube and of the velar valve.

29. In the free *Ichthyomyzon concolor*, by the use of carmine and water, a gentle current may sometimes be seen entering the mouth during the inspiration and passing out through the gills.

30. The teeth of the oral funnel of *Petromyzon marinus* are so arranged in concentric loops that when moved in radial lines by the action of the annularis muscle they lacerate every part of the surface with which the funnel is in contact.

31. The tongue of the attached *Lampetra wilderi* or *Ichthyomyzon concolor* may be thrust forward so as to bring its teeth in contact with the surface of its host and thus when the animal is feeding the lingual teeth aid those of the oral funnel in lacerating the tissue of the host.

32. When the tongue is thrust forward in these attached lampreys so that it no longer serves to maintain the vacuum in the mouth cavity by closing the cavity posteriorly, the semiannularis muscle is believed to contract and thus to maintain the closure

of the mouth cavity. In this way the lamprey is enabled to use the tongue in feeding without loosening its hold on its host.

33. After relaxation of the semiannularis muscle the food in the oral hood of the attached lamprey is believed to be pumped into the pharynx by the piston-like action of the tongue working in the mouth cavity and to be forced thence into the œsophagus by the contraction of the muscles of the pharyngeal wall.

34. The intestine of a single specimen of *Petromyzon marinus unicolor* taken in December from Cayuga Lake, N. Y., was found to contain not only blood but muscle, bone, the gill arch of a small teleost and other tissues, probably those of the host to which the animal had been attached. Gage's ('93) statement that this species feeds only on blood of the host is thus erroneous.

35. In *P. marinus*, *Lampetra wilderi*, and *Ichyomyzon concolor* there projects forward into the pharynx from the cephalic end of the united walls of the œsophagus and water tube a pair of jaw-like structures, the velar jaws.

36. The velar jaws are adjacent to the velar valves which guard the opening from the pharynx into the water tube and the two are supported by a continuous pincer-shaped cartilaginous frame work, actuated by muscles in such a way that closure of the velar valves approximates the velar jaws ; while opening of the velar valves separates the velar jaws.

37. When the attached lamprey is feeding, the velar valves close the entrance of the water tube and thus prevent food from entering the water tube and gill sacs. The velar jaws are closed and it is believed passive during this process.

38. The statement made by Günther (1853) and Abbott (1875) to the effect that the *free* lamprey feeds on fish and eggs or small invertebrates are not supported by the examination of stomach contents.

39. It may be possible for the lamprey to feed when free on minute forms or on somewhat larger animals siezed and held between the approximated halves of the oral funnel. The minute forms or the fragments resulting from the laceration of the larger forms might then be carried to the pharynx by a current of water entering the mouth.

40. If the lamprey feeds when free by means of a current of water entering the mouth it is possible that by a simultaneous closure of velar valves and velar jaws, food particles which would otherwise be swept into the water tube are held between the velar jaws. By the simultaneous opening of the velar valves and velar jaws a current of water from the mouth cavity into the pharynx might carry a food particle from the velar jaws into the open œsophagus. On the other hand, a current of water from the water tube might expel such a food particle through the mouth.

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THE CRANIAL AND SPINAL GANGLIA AND THE VISCERO-MOTOR ROOTS IN AMPHIOXUS.

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As my study of the central nervous system of *Amphioxus* must be interrupted for some months on account of other work, I will publish now a brief description of the cells of origin of the nerve components which constitute the dorsal roots.

Reviews of the literature on the nervous system of *Amphioxus* are given in papers cited below (nos. 3, 4, 5,) so that it will be necessary to speak here only of the work bearing directly on the elements to be described. Rohde (1) thought that the nuclei which he saw in the roots of the dorsal nerves indicated the presence of the equivalent of the spinal ganglia of vertebrates. On p. 199 he says: "Allenthalben liegen den sensiblen Nerven Kerne eingebettet, welche namentlich häufig bei ihrem Abgange vom Rückenmark auftreten. Sie haben genau dasselbe Aussehen wie die besonders in der Epithellage des Hirnventrikels häufig vorkommenden Nervenkerne und sind diesen sicherlich identisch, also nervöser Natur . . . Den Spinalganglien der höheren Wirbelthiere entspricht also bei *Amphioxus* eine Ansammlung nervöser Kerne." The author then reviews earlier comments on these nuclei. Neither Rohde nor earlier authors saw cell bodies or nerve processes belonging to them. Evidently the mass of nuclei described by Rohde is continuous with the small nests of ganglion cells mentioned by Hatschek (2). This author says: "Die dorsale Wurzel, welche bekanntlich keine Verbindung mit der ventralen eingeht, steigt nahezu in dem Winkel des Myoseptums gegen die Unterhaut empor und theilt sich dort in einen dorsalen und ventralen Ast. Kleine Nester von Ganglienzellen finden sich besonders an der Theilungsstelle des Nerven, z. T. aber auch schon in dem aufsteigenden Teile und auch in den Ästen. Der aufsteigene Teil ist daher als eine ausgezogene Wurzel zu betrachten und die Spinalganglien, welche wenig konzentriert sind, liegen in der Unterhaut (in unmittelbar Nahe

ihres epithelialen Entstehungsortes)." The methods used by these authors were inadequate to demonstrate the character of the cells to which they called attention and later authors working by special methods have denied the nervous nature of these cells.

Retzius (3) describes two types of cells within the spinal cord of *Amphioxus* which send fibers out in the dorsal roots. One type consists of small and medium sized bipolar cells transversely placed at either side of the central canal or extending across it. From one end of the cell a fiber enters the dorsal root. The second type consists of longitudinally placed bipolar cells from either end of which a fiber passes rostrad or caudad in the dorsal fiber bundles. One of the fibers arising from such a cell divides in T-form, sending a lateral branch into the dorsal root. After reviewing the relations of these cells Retzius says (p. 45): "Wo sind nun die *Spinalganglien*? Es giebt deren *keine*. In den sensiblen Wurzeln sucht man sie vergebens, sowohl innerhalb der Rückenmarksgrenze wie ausserhalb derselben. — In dem nächsten Verlauf der sensiblen Zweige trifft man weder einzellne Ganglienzellen noch Gruppe von solchen. Die Asnchwellungen, welche einige Autoren erwähnen, waren gewiss nur zufällige Bildungen. Ebensowenig konnte ich etwaige Stellvertreter, sog. 'Analoga,' der Spinalganglien entdecken; auch die von Rohde in die Wurzeln eingebetteten Kerne, die er geneigt ist als solche zu betrachten, sind meiner Ansicht nach nicht nervös, nicht 'Analoga' der Spinalganglien.

"Wenn also 'Analoga' oder richtiger Homologa der Spinalganglienzellen ausserhalb des Rückenmarks nicht nachweisbar sind, so bleibt die Frage unbeantwortet, ob nicht entsprechende Ganglienzellen im Innern des Rückenmarks vorkommen. Und man hat dann daran zu denken, ob nicht die beiden Reihen longitudinal angeordneter Ganglienzellen, deren Stammfortsätze nach geschehener Teilung (in T-form) in die sensiblen Wurzeln austreten, möglicherweise den Ganglienzellen der Spinalganglien entsprechen können."

Heymans and van der Stricht (4) described the development of the dorsal root by two rootlets, a dorsal cellular and a dorsolateral fibrous strand. With regard to spinal ganglia they say

(p. 16): "Si l'on examine très attentivement des diverses parties constituantes, aux différents stades de leur développment, de cette double ébauche radiculaire, nulle parte on ne constate une trace d'ébauche ganglionaire. L'étude de l'Amphioxus adulte, à l'aide de la méthode de Golgi, à l'aide du bleu de méthylène et à l'aide des méthodes ordinaires, ne révèle l'existence d'aucune cellule ganglionnaire proprement dite sur le trajet des racines dorsales, mais dévoile leur présence à l'intérieur du névraxe lui-même. Nous croyons donc pouvoir en conclure qu'il n'existe point de ganglions spinaux ni de ganglions crâniens sur le parcours des racines dorsales chez l'Amphioxus."

Dogiel (5) has described at length certain structures which he finds attached to the nerve rami just distally from the point of division of the dorsal roots into dorsal and ventral rami. The structures appeared in animals subjected for a long time to methylene blue in physiological salt solution. They were never stained by methylene blue dissolved in sea water. Dogiel was uncertain whether they may not have been artificial products due to immersing the animals in physiological salt solution. He was unable to make out their structure in preparations fixed and stained in various ways. He decided that they are normal structures, however, since they stain well with gold chloride and are visible in animals treated with osmic acid. The structures in question are rounded or pear-shaped bodies which are found in clusters of two, three or four attached to the nerve rami as berries are attached by their stems. The author concludes: "Sämtliche aufgezählten Thatsachen lassen sich am ehesten in der Weise auslegen, dass die beschriebenen Elemente Analoga von Spinalganglien darstellen, welche beim Amphioxus möglicherweise in einer embryonalen Entwicklungsform vorhanden sind. Eine endgiltige Entscheidung der Frage über die Natur dieser Gebilde ist jedoch natürlich nur dann möglich, wenn die Structur derselben und ihre Beziehung zu den Nerven sicherer bestimmt sein wird."

I can add with regard to these structures, first that they do stain by methylene blue dissolved in sea-water, and second that they are to be found along the course of the ventral ramus as far ventrally as the middle of the lateral surface of the body.

Although I have had them stained in a large number of animals they have appeared only in specimens which had remained a long time in a relatively strong stain or had been exposed to the air for a long time after staining, or both. In all cases the animals, although alive and reflexly irritable, were in an extreme state of weakness. I have never seen in these bodies stained with methylene-blue such a structure as would indicate that they are normal nervous organs. They always appear as pale, granular or structureless pear-shaped or balloon-shaped bodies attached by the small end to the nerve ramus. I do not see that any fact regarding these bodies suggests comparison with the spinal ganglia of vertebrates. Their form, their position, the fact that they appear stained with methylene-blue only when the animals are subjected to physiological salt solution or are kept in stain in sea water until they have reached a state of extreme weakness, all indicate that they are probably artifacts. From my own observations I should conclude that they are formed by the exudation of fluid from the nerve through a rupture in its sheath. The exuded fluid takes the form of a balloon, remaining attached by a stalk. The most favorable place for the formation of such exudation is in the angle between the dorsal and ventral rami and between two myotomes. They are formed also, but less often, far along the ventral ramus and even relatively far out along the dorsal ramus. If by physiological salt solution in which Dogiel stained his animals, he means a solution of sodium chloride of 0.75 per cent., more or less, this would be very favorable to the formation of such artifacts, since a physiological salt solution *for Amphioxus* must contain upwards of three per cent. of sodium chloride. The animals soon die in salt solutions of less strength. Dogiel states indeed that his animals died quickly in the physiological salt solution and that the structures described were brought to view by the stain only after three or four hours! Maceration must have been going on rapidly during all that time.

Finally, I have been unable to find these bodies in sections of animals well fixed and stained by various methods, or in specimens treated with osmic acid. The resemblance which Dogiel notes between these bodies and certain structures connected with the rostral nerves is not at all close. On the proximal portion of the first, second, third and fourth nerves of normal living animals are to be seen rounded projections or knobs which might be described as bud-like or as resembling an up-turned thumb. The greatest difference between these structures and the supposed spinal ganglia is that these stain in methylene-blue at the same time with those bodies which are connected with the end branches of the rostral nerves; that is, some hours earlier than the supposed spinal ganglia.

My own observations have been made upon animals stained in methylene-blue and upon sections prepared by the Golgi method and by a variety of hæmatoxylin methods after fixation in twenty per cent. formalin, Worcester's, Zenker's or Flemming's fluid.

In living animals stained with methylene-blue, both types of cells described by Retzius and the fibers from the transverse cells passing into the dorsal roots have been clearly and repeatedly seen. In two specimens also, many examples of slender bipolar cells were seen in the root of the nerve in the situation of the cells shown in Fig. 4. Each of these cells sent one process out along the nerve and one into the cord. When the cells in this position are examined in hæmatoxylin sections they are seen to be spindle-shaped cells with elongated nuclei and with a process from each end of the cell which can be followed for a considerable distance. The cells have very slender bodies with only a thin layer of cytoplasm over the nucleus. Except for the difference in size, which is more or less proportional to the difference in the size of the two animals, these cells are closely similar to the ganglion cells of the cutaneous and lateral line fibers in Petromyzon (*Lampetra Wilderi*, 6). Such cells are found in the root and undivided trunk of the nerve, in the proximal part of the dorsal and ventral rami, and also in the cord near the root of each nerve. The largest number of cells are found in the proximal part of the root and in the somewhat conical protuberance of the cord which gives rise to the root. In the so-called cranial nerves (I., II.) the large size of the roots and the somewhat more regular arrangement of the cells and fibers enable one to see these elements more clearly than in the trunk nerves. Comparison of horizontal and transverse sections shows that in both cranial and trunk nerves the cells are distributed throughout the thickness of the nerve root.

Sections by the rapid Golgi method show these cells and confirm the description given above. Cells of the following types are seen to send fibers into the dorsal roots. In the figures the several types are indicated by the arabic numerals which are used here in the text. (1) Bipolar cells near to or extending across the middle line of the cord, whose central processes go to the further side of the cord from the roots which the peripheral processes enter. The central process after reaching the opposite side of the cord either (*a*) enters the dorsal bundles without dividing, (*b*) divides in T- or Y-form into rostral and caudal branches, or (*c*) ramifies at once more or less profusely. The last mode of behavior is seen most often in my preparations. These cells mingle with the more centrally situated cells of the next type. (2) Bipolar cells within the cord more or less radially placed with reference to the nerve root, whose central processes remain on the same side of the cord as the roots which the peripheral processes enter. These cells may be situated anywhere within the area of a fan whose handle is represented by the nerve root and whose rays are represented by these cells and their processes. The bodies of the cells are situated among the bundles of root fibers as they turn for-

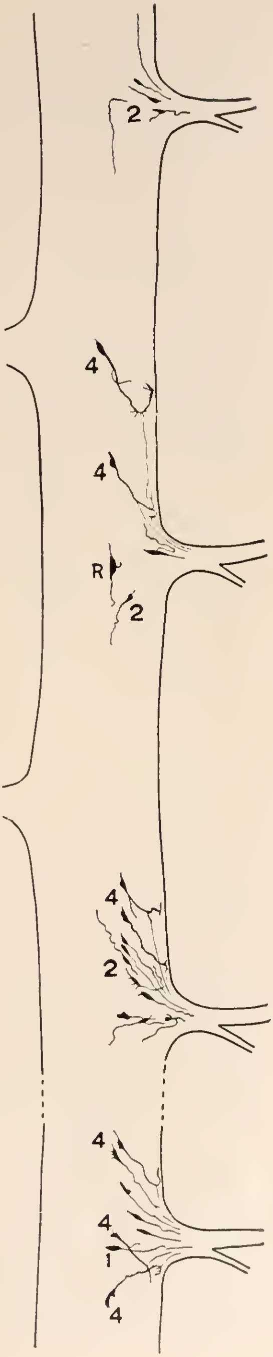

FIG. 1. A horizontal section of the nerve cord and dorsal roots. The parts above and below the dotted lines were drawn from adjacent sections of the same animal. The arabic numerals indicate the types of cells described in the text. *R*, longitudinal bipolar cell of Retzius. The smaller dorsal ramus of each nerve diverges caudally from the larger ventral ramus.

ward or backward in the cord, and the central processes run with the root fibers in the dorsal bundles of the same side. More cells of this type are impregnated than of either of the others but only enough have been drawn in the figures to illustrate their position. (3) Bipolar cells in the root or trunk of the nerve whose central processes are seldom impregnated far into the cord. Those that are impregnated enter the dorsal bundles of the same side. Many root fibers are impregnated which show no cells connected with them. These all run forward or backward in the dorsal bundles of the same side. They

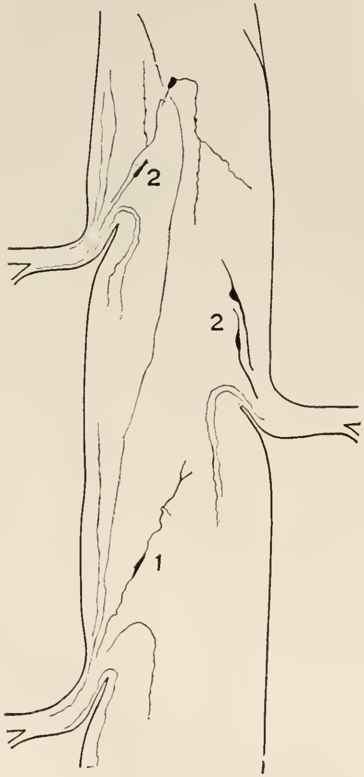

Fig. 2. Horizontal section of the cord between the tenth and eleventh dorsal nerves. The form of the nerve roots shown is characteristic of this region of the body. On the right side are two very coarse fibers with their ganglion cells.

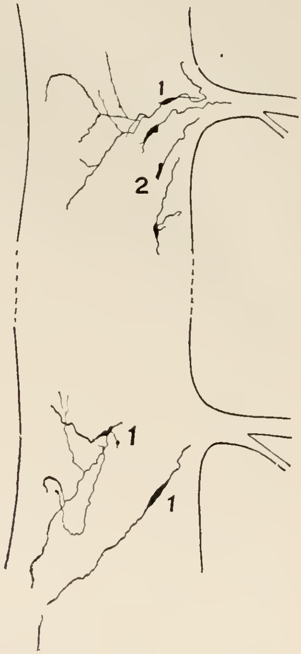

Fig. 3. Horizontal section showing especially fibers going to the opposite side of the cord.

are doubtless fibers whose cells are situated in the root or trunk of the nerve. As shown in Fig. 4, these cells are sometimes present in the dorsal and ventral rami, and it seems probable that they will always be found there. I have not been so fortunate as to have any cells impregnated far out beneath the epidermis in the position indicated by Hatschek, although such cells are readily seen in hæmatoxylin sections. (4) Irregularly pyramidal cells situated near the canal at a slightly more dorsal level than the pigment cells. These cells are usually provided with a single coarse process which runs to the surface of the cord where it ends in a few thick branches or in broad plate-like expansions against the limiting membrane. From some point in its course this thick process (dendrite) gives off a fine fiber which enters the dorsal root. Eight of these cells are shown in Fig. 1.

There can scarcely be any question of the homology of the cells of the first three types described with the spinal ganglion cells of vertebrates. The description confirms the account given by Retzius of cells within the cord sending fibers into the dorsal roots, but it shows that by far the larger number of such cells are situated where Retzius distinctly denied the existence of any nerve cells. They are the cells whose nuclei attracted the attention of Rohde and the earlier authors. The facts given by Retzius together with the discovery (7, 8) that the giant cells in the cord of teleosts are comparable with spinal ganglion cells have been considered as evidence that the spinal ganglia in vertebrates have been derived from the spinal cord. Now that the disposition of the ganglion cells in *Amphioxus* is more fully known it shows that this animal is not so different from vertebrates in this regard as was supposed. In *Amphioxus* the spinal and cranial ganglia form for each nerve an almost continuous mass extending from the central canal of the cord out into the root of the nerve to and beyond the division into dorsal and ventral rami. Thus it may be said that part of the ganglion cells in *Amphioxus* occupy a place within the cord which has been regarded as the hypothetically primitive position for vertebrates, while most of them occupy a place in the nerve roots which approaches the typical position for higher vertebrates. *Amphioxus* is, therefore, not quite primitive in this matter but rather approaches typical vertebrates.

One fact makes it seem probable that there is a movement peripherally of the spinal ganglion cells in *Amphioxus* after the period in the ontogeny when the longitudinal fibers of the cord are formed. Many of the fibers adjacent to the roots are bent out far into the root where they recurve and pass on in their former course within the cord. A slight case of this is shown in Fig. 4. In many cases such recurved fibers extend much further out into the nerve roots, and the number of fibers affected in this way is so great that the bulging laterally of the cord toward each root is very striking in horizontal sections. Occasionally some of the giant fibers are carried toward a root until they seem as if they were about to enter it. I can think of no other cause for this curving of fibers out into the roots except the possible active migration of the ganglion cells.

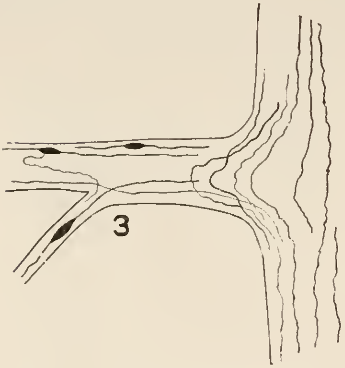

FIG. 4. Horizontal section of dorsal root showing ganglion cells in the trunk and rami. Combined from two sections.

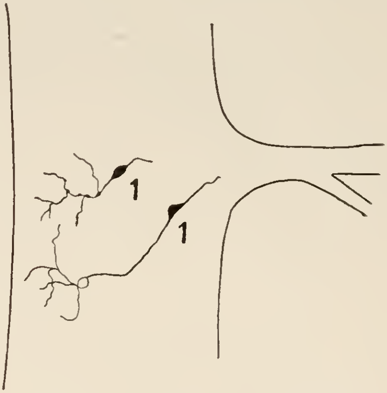

FIG. 5. Two cells whose neurites ramify at once on the opposite side of the cord.

The place of branching of the dorsal roots into dorsal and ventral rami is of some interest. The branching seldom takes place close beneath the dermis as Hatschek describes it. On the other hand, the division of the root near the cord and the separate origin of dorsal and ventral rami directly from the cord are of more frequent occurrence than Rohde states, and are not confined to the anterior end of the body. The separate origin of the rami may be seen in the case of one or several roots in a considerable majority of specimens. Every possible gradation is to be found between this and the manner of division described by Hatschek. The typical place of division is about half way between the cord and the dermis. This is not only the mean between two extremes, but it is the place where the rami separate in the great majority of cases. In proportion as the division into rami occurs nearer the cord, a greater number of ganglion cells are found in the rami. The argument of Fürbringer (9, p. 646) that the lateral musculature of *Amphioxus* corresponds to the mesial portion only of that of craniates, based upon Hatschek's description of the spinal ganglia of *Amphioxus* outside the muscles, is not supported. The greater part of the spinal ganglion of *Amphioxus* is situated mesial to the muscles as in craniates.

It is hoped that the cutaneous and visceral sensory fibers in the dorsal roots may be distinguished, the position of their respective ganglion cells determined, and the central course of each component traced. The more general facts regarding these points seem now to be clear.

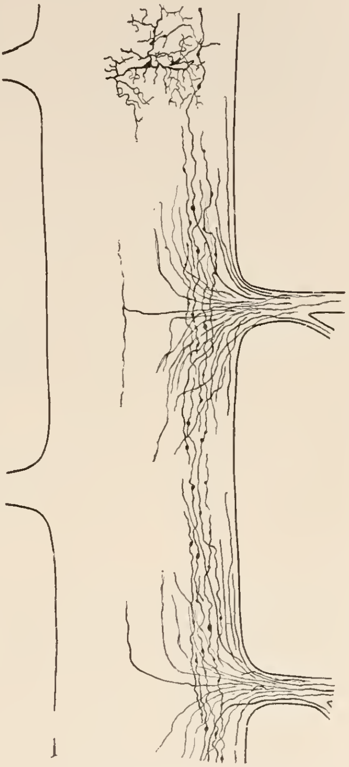

Fig. 6. Horizontal section showing the spreading of the sensory roots into mesial fine-fibered, lateral coarse fibered bundles and the formation of the dorsal compact bundle. The fibers of the last bundle are strongly varicose. At the upper part of the figure is shown the end-branching of a coarse sensory fiber from the root opposite.

The great majority of the fibers of the dorsal roots are fine or medium sized; a few only are very coarse. The disposition of the fibers in the cord can be seen best in horizontal sections prepared by the Golgi method. As they enter the cord the fibers spread forward and backward and many pass to or across the middle line, diverging more or less. The fibers which remain on the same side separate into two ill-defined bundles, of which the one nearer the median line consists of the fibers. The medium and coarse fibers are situated laterally. In a high focus, somewhat above the root of the nerve, a distinct bundle of fibers is seen running along dorsal to and independent from the spreading fibers of each nerve. Some fibers of each nerve, however, enter this bundle. The bundle consists of medium coarse fibers and is situated at the surface of the cord between the mid-dorsal line and the nerve roots. Its position is shown in Figs. 6 and 7. This bundle is distinctly seen in hæmatoxylin sections but has a very different appearance after different fixing agents. It is not well fixed in all fluids. In Zenker's fluid it contracts and the fibers become aggregated into a dense mass which is surrounded by an open space. In twenty per cent. formol the bundle has the appearance of poor fixation with swelling. It appears as a lightly stained reticulated area in which the fibers are not sharply visible. In both these fluids the remainder of the cord seems to be well fixed and stains well. In Worcester's fluid, which has

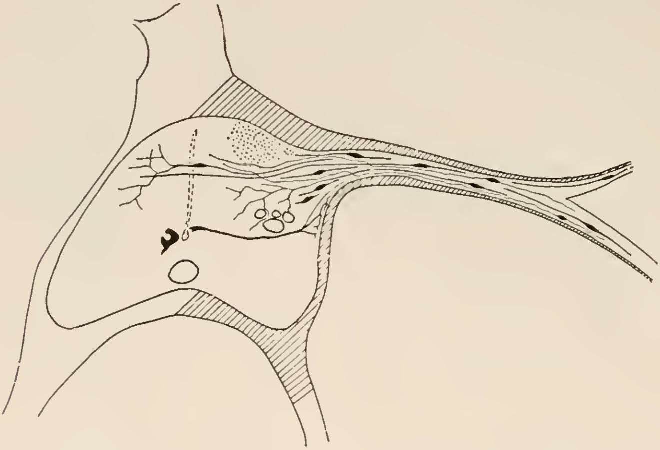

FIG. 7. A diagrammatic transverse section of the nerve cord and a dorsal root. The sheath of the nerve cord is marked with oblique lines. The right lateral group of giant fibers, the mid-ventral fiber and one pigment cell are shown. The stippled area shows the position and size of the compact dorsal bundle of fibers as it appears in the middle region of the body. The disposition of the coarse and fine fibers and their ganglion cells and the position of one viscero-motor cell are shown.

remarkable penetrating qualities and is a faithful fixing agent, there is no shrinking or swelling and the fibers are well fixed. The bundle appears as an area of rather coarse fibers which take a deeper stain than the remaining fibers in the dorsal region of the cord. The bundle extends throughout the whole length of the nerve cord, at least from nerve II. well into the tail region where it grows very small. The bundle is very noticeably larger on the right side than on the left and on both sides the bundles increase in size toward the head end, perhaps because the majority of the fibers are ascending. In the head region the bundle of the right side is augmented considerably by fibers from each of the nerves VI.–III. inclusive. These fibers run forward and mostly leave the bundle within one segment, for the nerve is small immediately behind each of the nerves mentioned. In brief, the sensory roots form three tracts in the dorsal part of the cord, a diffuse mesial tract of fine fibers, a diffuse lateral tract of coarser fibers and a compact dorsal superficial tract of coarse fibers. The first and second mingle more or less with one another and with fibers of other kinds running ventral to them. The ventral limit of the dorsal tracts taken as a whole is roughly marked by the lateral group of giant fibers.

The very fine fibers seem never to be connected with ganglion cells within the cord, but some of them do have their cells in the nerve trunk. They run for a comparatively long distance in the cord without dividing. Since the visceral sensory fibers in vertebrates are fine, the hypothesis presents itself that these are the visceral fibers in *Amphioxus*. With this their position near the dorsal raphe is consistent. Evidently the visceral fibers do not enter the well defined bundle of coarser fibers ; for, if they did, that bundle should be very large on the left side in the head region where all the visceral surface is supplied by nerves of the left side. The bundle is small on the left and larger on the right.

The coarser fibers comport themselves in a variety of ways on entering the cord. (In speaking of coarse and fine fibers of the dorsal roots one must compare the central processes of ganglion cells with central processes and peripheral processes with peripheral processes and in order to do this one must know where the ganglion cell of a given fiber is located.) Speaking then of the coarser central fibers, some of them ramify at once on entering the cord, others divide in T-form and run in the dorsal bundles of the same side, others enter the dorsal bundles without dividing, others go to the opposite side of the cord and either ramify at once or run in the dorsal bundles, with or without bifurcation. Altogether, the number of coarser fibers which ramify near to the root from which they come is striking. If these are cutaneous fibers a striking physiological fact is explained, namely the relative independence of the segments in locomotion ; otherwise expressed, the small number of segments necessary to perform the typical swimming movements. A short piece of the tail end can swim well and behaves much as a whole animal does ; and this for many days together.

To illustrate by a complex movement, normal animals in a shallow dish persistently put their heads up over the edge of the dish and then by swimming round the dish and pushing against the edge succeed in wriggling over, if the edge is not too high. The isolated tail makes the same persistent and apparently purposeful efforts when the dish is very nearly full of water. When an animal is so macerated that *all* the tissues except the notochord are gone from the middle of the body, the two parts perform typical swimming movements but each with an independent rhythm. This retention of the power of coördinated movements by a few isolated segments is perhaps connected with the large number of cutaneous fibers which have a short course in the spinal cord. This makes it possible for the muscles to be reflexly controlled by stimuli received at the surface of the body in the same or adjacent segments.

Finally, a few coarse fibers whose ganglion cells are in the nerve trunk go to form the definite bundles described above. Since these are chiefly ascending fibers which have a long course in the cord, the bundle may be compared with the dorsal tract of the same description in vertebrates, the tract of Goll. This is therefore probably the first tract to appear as a definite bundle in the vertebrate nervous system.

The fourth type of cells described in this paper are the viscero-motor cells. In position they correspond to the visceromotor column as it is known in fishes and other vertebrates.

They are lateral to the ventral parts of the canal. They retain primitive characters in that the cell body is adjacent to the canal and that there is a single large dendrite which extends to the periphery of the cord. The origin of the neurite from the dendrite at some distance from the cell body is perhaps also a primitive character so far as the vertebrate nervous system is concerned. With respect to the disposition of these cells along the cord it is evident that they form a more or less complete column and that the neurites often run for some distance in the lateral tracts to reach their roots (Fig. 7).

As our knowledge of the nervous system of *Amphioxus* increases its effect is to oppose the tendency of recent years to minimize the relationship between *Amphioxus* and vertebrates, to consider *Amphioxus* as far removed from typical vertebrates and more closely related to invertebrates rather low in the scale. While there is great significance in the similarity of the nephridium (10) and eye-spots (11) of *Amphioxus* to those of some worms, the close relation of the nervous system of *Amphioxus* to that of vertebrates has also an unquestionable significance. *Amphioxus*, indeed, contributes more toward bridging over the gap between vertebrates and invertebrates than has usually been supposed.

The nervous system of *Amphioxus* agrees with that of lower fishes in the following respects :

(*a*) It is dorsal, hollow, and has separate dorsal and ventral roots of definite composition. The canal has an enlargement at the anterior end, the brain ventricle.

(*b*) The dorsal roots consist of general cutaneous, visceral sensory and visceral motor components. They contain also in the head region fibers of special sense organs (olfactory or gustatory ?).

(*c*) Both kinds of sensory fibers have ganglion cells which are situated either within the cord or in the root of the nerve in essentially the same position as the spinal ganglia of vertebrates.

(*d*) The two kinds of sensory fibers on entering the cord form dorsal tracts similar to those in vertebrates. Many cutaneous fibers show the bifurcation characteristic of these fibers in vertebrates.

(*e*) The viscero-motor cells are situated as in vertebrates dorsal to the somatic motor cells, lateral to the ventral part of the canal.

(*f*) The nerve cells retain the position and characters which are typical in the embryos of vertebrates and which are seen in certain parts of the brain of many fishes.

(*g*) The ventral roots arise separately and remain independent. They are true somatic motor nerves.

We have here nothing else than an essentially vertebrate type of nervous system. At the same time there are good indications of a truly primitive, unspecialized condition. In addition to the facts given under *a*, *c* and *f*, there may be mentioned :

(*h*) The total absence of certain specialized structures which characterize all vertebrates, namely, hair cells responding to vibrations in fluid (neuromasts or acustico-lateral organs), and retinal visual organs. (The morphology of olfactory and gustatory sense organs is yet in an uncertain state.)

(*i*) The presence of simple light-percipient organs within the central nervous system, which have apparently been retained from the worm-like ancestors of *Amphioxus*.

(*j*) The very slight development of the brain.

These facts speak eloquently against the supposition that *Amphioxus* is the result of a process of degeneration from any form which had reached a higher degree of specialization, such as selachians or other fishes. Similar facts have been brought forward (6, p. 73) to show the primitive character of the cyclostome brain. We cannot suppose that specialized structures in the nervous system once possessed by the ancestors of *Amphioxus* have been nicely pruned back and reduced in the process of degeneration ; nor can we believe that true nephridia and helminthine eye-spots should reappear in a degenerated species whose ancestors in the course of their evolution had lost these very organs. The straightforward interpretation of the nervous system supports the view that *Amphioxus* and Cyclostomes are the lower branches of the vertebrate phylum.[1]

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[1] The Worcester's fluid spoken of in the text is as follows :

40 per cent. formalin	10 parts,
Distilled water	90 "

Saturate this with sublimate ;
Add glacial acetic to make 10 per cent.

This work has been done at the Smithsonian table in the Naples Zoölogical Station. I wish to acknowledge my great obligation to the Institution for the opportunities thus afforded, and to express my best thanks to the officials of the zoölogical station for the excellent facilities provided.

NAPLES, January 25, 1905.

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'02 On the Structure of the Excretory Organs of Amphioxus. Part I., Q. J. M. S., Vol. 45, 1902.

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'98 Unterschungen über die Organe der Lichtemfindung bei niederen Thieren. IV., Die Sehorgan des Amphioxus. Zeit. f. wiss. Zool., Bd. 63, 1898.

PAULINELLA CHROMATOPHORA.

WILLIAM A. KEPNER.

In volume 59 of the *Zeitschrift für wissenschaftliche Zoölogie* Lauterborn has described an interesting new Sarcodin, which he named *Paulinella chromatophora.* During the months of December, 1894, January and February, 1895, he found and studied 200 individuals whose generic and specific characteristics he gave in the following:

"Genus *Paulinella.* Shell elliptical, sack- or flask-shaped, in transverse section circular, composed of five rows of six-sided, silicious plates, mouth of shell elevated upon a neck, very narrow, in transverse section a lengthened oval. The protoplasmic body does not completely fill the shell cavity; nucleus spherical, rather large, with a reticular structure, situated in the posterior part of the body; contractile vacuole in the anterior third of body. Pseudopodia long and slender, never anastomosing.

"*Paulinella chromatophora.* With the characteristics of the genus. Contains one or mostly two conspicuous sausage-formed chromatophore-like bodies of a blue-green color. The reception of food not observed, nutrition, therefore, apparently holophytic with the aid of the 'chromatophores.'

"Length of shells: 0.020–0.030 mm., width: 0.015–0.020 mm., diameter of chromatophores: 0.003 mm.

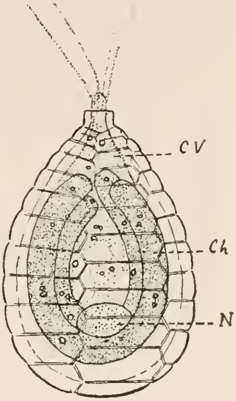
"Habitat: stagnant water of the Rhein near Neuhofen under diatoms in company with *Amœba, Difflugia, Englypha, Gromia mutabilis* Bail, etc."

So far as I know this form has not since been discovered. I wish to record it hereby for the United States. The specimens studied met the requirements of Lauterborn's paper so well that I am not justified in giving the details of the anatomy. For these the reader is referred to Lauterborn's account.

Mr. W. G. Lapham, of this place, discovered and recognized, on November 1, 1904, a single individual of *Paulinella chromatophora* in water taken from an oozy bank near Afton, Va. In December the writer found specimens from a spring-pool at Charlottesville, Va. They are mud-loving creatures and are found in the sediment upon dead leaves in company with many *Mallomonas Plosslii* Perty, some *Amœba radiosa*, and *Acanthocystis* sp. and an occasional desmid.

The shells in our specimens were somewhat thicker than Lauterborn describes.

The bulk of the protoplasmic body varies, but it never fills completely the shell cavity. In one individual the protoplasmic body was contracted into a sharply defined spherical mass, which lay at the base of the shell cavity. Before pseudopodia appear a rounded neck of protoplasm is extended through the narrow neck of the shell. And then suddenly one or more pseudopodia are thrust out from this protruding bit of protoplasm. For a moment the pseudopodia remain quiescent; but they are usually oscillating or slowly waving like a flagellum. It is strikingly interesting to see these pseudopodia function as primitive flagella.



An isolated individual kept in a moist chamber at living-room temperature from January 5 to January 18 showed on the latter date *two large horse-shoe shaped chromatophores* which lay side by side with their ends directed towards the mouth of the shell. On January 5 this individual had but one horse-shoe shaped chromatophore. The specimen lived in this condition one week longer when it was accidentally killed. It remains to be seen, therefore, whether these two large chromatophores were developed as an effect of the artificial environment, or whether they were a step preparatory to cell-division, or a mere variation which will be more frequently met with when greater numbers are found.

We have not counted the individuals found. They are plentiful and can be found with comparative ease in water from the spring-pool by Preston Heights, Charlottesville, Virginia. We have succeeded in keeping *Paulinella chromatophora* alive in small aquaria and moist chambers at living-room temperature for periods as long as three weeks.

University of Virginia,
February 11, 1905.

TORSION OF THE CRUSTACEAN LIMB.

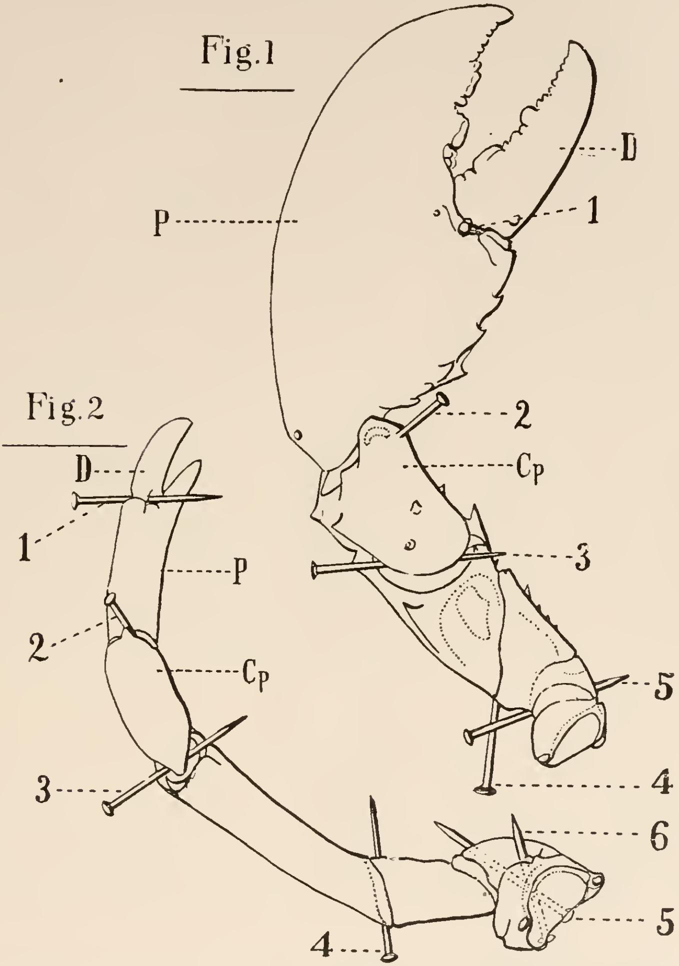
FRANCIS H. HERRICK.

The large double claws or chelæ, which are so conspicuous in many of the higher crustacea, have not failed to attract the attention of naturalists, both on account of their remarkable regenerative phenomena as well as for the aid which they afford to systematic zoölogy. It is therefore the more surprising that attention has never been called to a striking instance of torsion in the great chelipeds of two of the best known forms — the crayfish and the lobster.

If we examine the appendages of either animal from above (Figs. 1 and 2) it will be seen that while in the great claws the dactyles face and open inwards and therefore in a nearly horizontal plane, all the smaller chelæ open upward and outward in a plane which is nearly vertical. In the lobster, however, at the time of birth (Fig. 4) the three pairs of chelate legs, great and small, all have the same form and position, that is, the claws, which are laterally compressed, all open vertically with an inclination outward. It therefore follows that the position of the great 'forceps' has been reversed by rotation through 90°, in consequence of which their inner or anterior faces have become their under sides (Fig 3). The metamorphosis has been reduced to such a degree in the crayfishes that the young are hatched with the essential characteristics of the adult form. If therefore a torsion really occurs in the limbs of these animals, it must be sought in the egg.

In the true crabs, or such representative forms as *Callinectes* and *Carcinas*, the claws open outward, as in the larval lobster, and the outer or posterior face, corresponding to the upper side of the chela of an adult crayfish, tends to become the under side, and is deficient in pigment.

In the crayfish again, about one quarter of the weight of the animal is represented by the great chelipeds, while their proportional weight in the lobster is one half. The acquisition of size and strength in these limbs, and in *Homarus* the remarkable differentiation into toothed and crushing forceps of either right or left sides, has been accompanied by a permanent torsion, which has chiefly affected the carpodite, or fifth joint (as counted



FIGS. 1 and 2. Left first and third chelipeds of *Homarus americanus* in natural position, seen from above. Pins 1–5 are inserted close to the hinge joints of the successive segments in each limb, to illustrate the degree of torsion which the great chelipeds have undergone. Compare the position of the hinge-joints 1 and 2, Fig. 1, with that of the corresponding joints in Fig. 2. *Cp*, Carpodite ; *D*, dactyle ; *p*, propodite ; *1–6*, pins inserted in hinge-joints.

from the base — *c. p.*, Figs. 1 and 2). As I shall later show however, this twisting of the limb is entirely independent of the form or weight of the claw. Meantime the eight slender legs, which follow the larger pair, have remained stationary, and have retained their larval form and position.

In the larval lobster the first pair of chelate limbs are prehensile organs solely, by which the food is seized and transferred to the mouth-parts. Later, when the double claw is fully developed, and is provided with either crushing tubercles, or rows of interlocked tooth-like spines, the prey is usually crushed and torn in pieces before its delivery at the mouth by the smaller claw-feet. The toothed or "quick" claw is primarily used for striking and capturing the prey.

The rotation of the chela, in the lobster, is completed at the fourth molt, which marks the most surprising leap in the whole history of development, for at this time the larval swimming organs are laid aside and as Prentiss * has shown, the antennulary pockets or balancing organs come into play, when, for the first time the miniature lobster swims steadily, and in an upright position. At this crisis new instincts also arise ; when the lobsterling swims rapidly at the surface, the great claws are directed forward and held close together, but if its pugnacity is aroused, it assumes the well known attitude of defense displayed by an adult animal.

In dead lobsters or crayfishes the large claws, in response to gravity, lie perfectly flat, but this position cannot be assumed in life unless the muscles of the limb are completely relaxed. In the common-position of wariness and defense, the claws are upraised, and tilted obliquely inwards, so that their tips rest close together on the bottom.

In both *Cambarus* and *Homarus* the disposition of pigment is a more striking index of the rotation of the limb than changes in its form, and even more than in the crab have the under sides or what were once the anterior faces of the claws become differentiated by the absence of color. The mottled green pigments which are so thickly spread over the whole upper surface of these animals are completely lacking on the under sides of the big claws.

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* "The Otocyst of Decapod Crustacea : Its Structure, Functions, and Development," *Bull. Mus. Comp. Zoöl.*, Vol. XXXVI., 1901.

The large forceps of either side differs in still other respects from the more primitive type-form preserved in the slender feet. Thus the lower margin of the weaker claw of the small cheliped is incurved, as contrasted with the rotund or convex outlines of the big chelæ, a condition probably determined by the greater muscular development of the latter.

Fig. 3

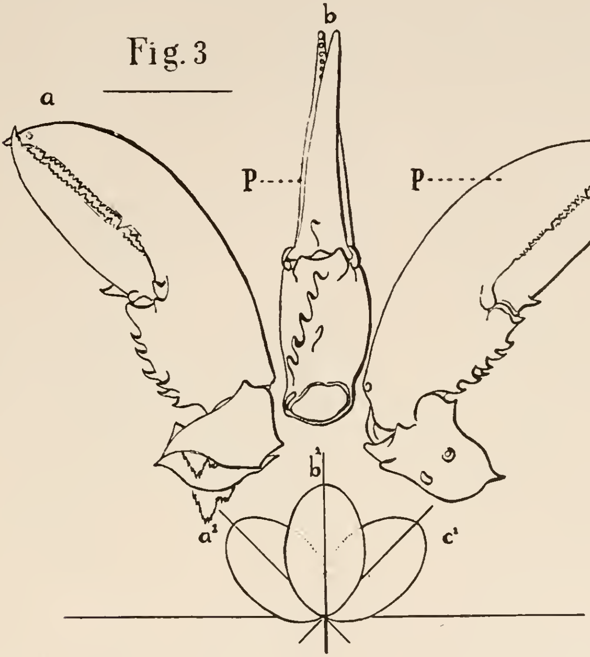

FIG. 3. Illustrating successive positions (*a*, *b*, *c*) in the rotation of the great claws of the lobster, and further by the vertical projection (a^1, b^1, c^1), shown below. In *a*, a^1, which represents the normal position in the larval lobster and adult crab, the upper or anterior surface of the claw becomes, in the adult lobster the underside *c*, c^1. *P*, propodite.

In Figs. 1 and 2, which represent the first and third claw-feet of the left side as seen from above, with the natural fore-shortenings, pins are inserted at successive hinge-joints, to illustrate the various degrees of torsion which parts of the limb have undergone. The joints mainly affected are the carpodite and propodite (*Cp*, *P*), and the extent to which they have been twisted may be measured by a comparison of pins (or hinge-joints) marked 1 and 2 in the figures. This phenomenon may be compared in a general way with the remarkable changes which occur in the metamorphosis of the flat-fishes, where the whole body is more or less completely involved. It seems to be confined to the closely allied Homaridæ (*Homarus*, *Nethrops*), Potamobiidæ (*Cambarus*, *Astacus*) and Parastacidæ (*Parastacus Astacoides*) of Huxley, besides the Paguridæ, or hermit crabs, the Galatheidæ,[1] or Spanish lobsters (*Galathea*, *Munida*), embracing many deep sea forms, which with a number of smaller families are often united into the larger division of the Anomura. In all these forms the dactyles of the large claws appear to open inwards, and although in many cases the smaller feet are non-chelate, or the metamorphosis is abbreviated or at present unknown, we may safely assume that their present state has arisen from a primitive condition such as we find illustrated in the first larval stage of *Homarus.*

That the modification in question was of very ancient origin does not admit of doubt. It was already perfected in the Erymoid crustacea which inhabited the Liassic seas, unless naturalists are wholly wrong in assuming that these primitive macroura were the ancestors of the modern crayfishes and lobsters.

According to Huxley[2] forty species of Eryma have been described from the rocks of the middle Lias up to the Jurassic (Purbeck to Inferior Oolite) formations. In *Eryma modestiformis* of Oppel, the claws open inwards, and the stalked eyes are relatively very large, as in an adolescent lobster.[3] After the death of such an individual as Oppel depicts the large claws would lie flat as in a dead lobster, but had no previous torsion in the limbs of this species taken place, the relaxed claw should have turned the dactyles outward as it does in crabs.

It is interesting to find that in the somewhat less primitive *Eryon arctiformis*, from the Jurassic slates of Solenhofen, a very decided approach to the brachyurans, or true crabs is seen in the short tail, the broad carapace armed with marginal spines, as well as in the slender antennæ and other appendages so far as they are known. Very significant also is the fact that as in the modern crab the claws open obliquely outward.

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[1] Kinnaman has figured five species of *Galathea*, in all of which, excepting *G. Andrewsii* the large chelæ open inwards, so his drawing is doubtless faulty in this respect. See "A Synopsis of the Britannic Spanish Lobsters and Schrimps," *Proc. Roy. Irish Acad.*, Vol. VIII., Dublin, 1862.

[2] "On the Classification and Distribution of the Crayfishes," *Proc. Zoöl. Soc. London*, 1878, and "An Introduction to the Study of Zoölogy," illustrated by the Crayfish. New York, 1893.

[3] See p. 163 and plate 8, "The American Lobster," Bull. U. S. Fish Commission, Washington, 1895.

In the homarine *Hoploparia*, found in the Cretaceous and early Tertiary periods, the form of the large chelipeds renders it highly probable that a torsion of this limb had already taken place. At all events this phenomenon has a very ancient history, and is probably older than autotomy which precedes the regeneration of certain limbs, and which in the most perfect cases is dependent upon a fusion of the second and third joints. In the lobster this fusion does not occur until after the fifth molt.

Fig. 4

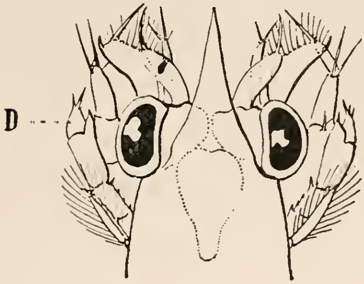

Fig. 5

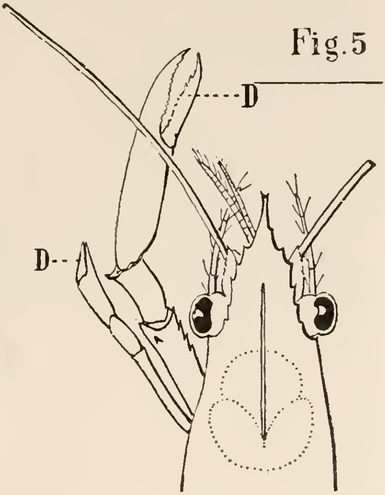

FIG. 4. First larva of lobster from above, showing the outwardly opening claw of the first pair of chelipeds. *D*, dactyle.

FIG. 5. Fourth larva or lobsterling stage of the lobster, showing the big claw-feet, in which torsion is complete, opening inward, toward the middle line of the body. *D*, dactyle.

II.

When we attempt to answer the question — How could a torsion of the crustacean limb be effected? — the results are not very reassuring upon either Lamarckian or Darwinian principles. The conditions of the problem seem to be very simple, and can at least be stated with a certain degree of exactness.

The segments of the limb mainly affected (Figs. 1 and 2) are the fifth and sixth (*P* and *Cp*, Figs. 1 and 2) which move on hinge joints. Each has a hard tubular shell, and lodges two muscles, a flexor and extensor, the fibers of which originate on the inner surface of the shell, and are inserted on tendons which engage with the next or distal segment, the mobility of the limb being further secured by soft interarticular membranes, and a series of hinge or peg-and-socket joints, set in different planes (Figs. 1 and 2). When the flexor muscle, let us say, of the carpus or fifth joint contracts, the huge claw moves on its hinge and is drawn upward and inward, as a door might be opened by means of cords, worked at a distance: at the same time the fibers react on the inner surface of the tubular shell, but the hinge-joint is fixed, and no conceivable contraction of such fibers can convert a straight pull into a twist. There is no room for the shifting or migration of the muscles, but this would not affect the conditions one way or another. Torsion has not occurred as a result of the normal movements of the limb.

It is to be noticed that the rotation of the claws occurs in larval life before the chelæ attain their full development, but if in the course of the evolution of these forms the increasing weight of the claws could have had any effect upon their ultimate position, it should have turned them in the opposite direction, because they lean outward in the larva. The theory that the effects of strain or use are inherited is therefore incompetent to account for the modification which exists.

In the present case it seems equally impossible to apply the selection theory of Darwin, and it would tax the imaginative faculty to determine wherein the chela of a crayfish was better adapted for prehensile purposes than the claws of hundreds of prawns, or how the toothed claw of the lobster excelled in this respect the pincers of the blue crab, *Callinectes*, or as a weapon of offence, the slashing, sabre-like, out-turned dactyles of *Alpheus heterochelis*, and of many another species of this large genus. I refer only to the power and ease of seizing, and not to the muscular force or to the armature of the claws itself, which is another question. But the height of the difficulty is not yet reached, for upon the selection theory, the torsion of such limbs must have arisen gradually, through successive fractions of a degree, until the member had moved through a quadrant of arc ; furthermore we are required to believe that each successive position was so much more favorable than the last, that forms showing such as well as possibly other correlated variations would outstrip their competitors, and alone leave descendents. As already suggested, the problem is somewhat analogous to the migration of the eye in the metamorphosis of flat-fishes, attempts to explain which in terms of the selection theory have not been very successful.

The difficulty of the problem is not lessened when we reflect that the slaughter of the young in a form like the lobster, takes place on a tremendous scale and is of the most indiscriminate character, before the prehensile organs are fully developed. We can only *guess* at the causes of such a variation. Its great antiquity, dating from as early at least as the Jurassic period, and other considerations already touched upon, render it highly probable that it first arose as a discontinuous variation. That it was not indispensable, is suggested by its erratic distribution at the present day.

Biological Laboratory, Western Reserve University,
February, 1905.

THE PHYSIOLOGY OF LOCOMOTION IN GASTEROPODS.

A Reply to A. J. Carlson.

DR. HERMANN JORDAN,
(University of Zürich).

In No. 2 of Vol. VIII., of this Bulletin, A. J. Carlson publishes a note on locomotion in gasteropods. He, at first, tries to show that my opinion concerning the mechanics of locomotion in *Aplysia*[1] is wrong. On page 87 he says: "Jordan (1901) rejects the theory of 'extensile Muskulatur' in accounting for the locomotion in the marine gasteropod *Aplysia*, and ascribes the relaxation or extension of the longitudinal muscle of the foot to the pressure of isolated bodies of the visceral fluid or blood. As evidence Jordan points to small reservoirs or lakelets of plasma in the strongly contracted foot. These lakelets are constricted off from the visceral cavity by the contraction of the muscular septa. A body of liquid thus cut off from the visceral cavity may serve to produce extension of the longitudinal muscle at its anterior border by the force of contraction of the oblique and transverse muscles at its posterior end. In this way we would have as many isolated bodies of blood being gradually pushed from behind forwards in the foot as there are areas of relaxation on the sole of the foot. The presence of isolated bodies of liquid in the strongly contracted foot is not a sufficient evidence that they are the factors in producing the waves of locomotion, as similar isolated bodies of liquid are also found in the musculature of the contracted mantle (*Aplysia Pleurobranchæa*). . . . Simroth and Jordan missed the true explanation by not taking into account the part played by the musculature of the dorsal and lateral walls of body cavity."

Carlson then describes a mode of locomotion, which he observed only in *Helix dupetithouarsi*. Instead of the ordinary locomotion by waves in the foot, this snail is able to lift up its head and to push it forward ; it repeats the same movement with the rest of its body, forming in this way two or three waves of the whole body. It is unnecessary to continue the explanation of this mode of locomotion : it is identical with the locomotion in many worms and caterpillars. I say identical, because it is immaterial how many waves there are.

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[1] *Zeitschr. Biolog*, XLI., p. 196.

"Nevertheless," Carlson says, at the very end of his note, "the peculiar mode of progression in the snail just described is probably only an exaggerated form of the ordinary locomotion."

I am sorry to be obliged to declare that Carlson did not know or understand my publication at all. Therefore I must repeat the passages in question, and I will try to translate them into English, in order to prevent a second misunderstanding.

Page 199 (*l. c.*) I say : The movement of the foot is formed by waves. To understand this kind of movement we must imagine the sole divided into several parts (Fig. 1). The wave begins through the extension of the first division *AB* in the direction *A*, and by adhering to the ground at *A*. Then the same part contracts, so that *B* approaches *A*. At the same time *BC* leaves the ground, extends, until *B* adheres, then *BC* contracts, *CD* extends and *C* adheres, etc. In this may we have waves advancing — when the foot is lifted from the ground — from the anterior to the posterior part of the body. (In *Helix*, *Limax*, etc., the waves advance in the opposite direction.) All these waves must be combined with appropriate adhesion in order to produce locomotion in the posterior-anterior direction.

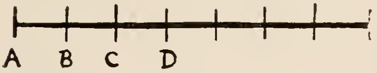

Fig. 1.

The snail is able to change this mode of locomotion spontaneously, *thus, for instance, Aplysia can execute movements similar to caterpillars of geometridæ* (Spannerraupen). I think this explanation should suffice, *for this mode of locomotion is known* (the mode now described by Carlson for *Helix*). In part 3 of the introduction of my work, page 200, I try to explain how extension is possible. This, I think, is the only part that Carlson read ; perhaps without understanding it. For in this chapter there is almost no question of waves of locomotion. The whole snail as well as its single parts is able to extend completely, therefore without coincident contraction of other muscles. This question was studied with pieces of the musculature, and I found that the extension of the relaxed muscle was performed by means of the pressure of the blood. *Under the conditions I observed*, the blood had been pressed into lacunæ beneath the snail's skin (*l. c.*, Pl. II.), and I showed that it was pushed back into the musculature by the extended collaginous tissue, extending it in this way. These lakelets may *sometimes* be completely "isolated."

Concerning the waves of locomotion I say, p. 236: when contraction takes place, water ("blood") is driven into the confined part, which thereby is extended; by this extension tonus is diminished, etc. It will be seen, that I never spoke of "as many isolated bodies of blood being gradually pushed from behind forwards in the foot as there are areas of relaxation on the sole of the foot."

Where is the difference between my opinion and that of Carlson?

Carlson thinks that every extension is produced by blood and organ-pressure, following the contraction of other muscles. According to my opinion, this mode is assumed in normal locomotion, *while the lakelets produce complete or partial extension.* (Perhaps first extension in locomotion also.) Carlson thinks that the special kind of locomotion is nothing else than an exaggerated normal one, consequently also produced by contraction of the "musculature of the dorsal and lateral walls of the body cavity."[1] *This opinion is wrong because, if this musculature is removed, the foot is able to produce normal locomotory waves.*

In conclusion: The opinion Carlson ascribes to me, is in reality not my opinion. My work of 1901 contains all that Carlson publishes as new in 1905, so far as the latter is true. The two modes of locomotion are quite different: in the normal one the blood, which is contained in the lacunæ (not in isolated lakelets!) of the foot, extends the musculature; in the fast one this rôle is performed by the contents of the body cavity, with the aid of the muscles of the dorsal and lateral sides of the body.

Zürich, March, 1905.

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[1] This ought to be regarded as the chief result of Carlson's note, because otherwise he would have done nothing to explain *normal* locomotion.

Vol. IX. *August, 1905.* *No. 3*

BIOLOGICAL BULLETIN

IMMUNITY AND ADAPTATION.[1]

LEO LOEB.

Various writers, in discussing the phenomena of adaptation, included among them the fact that in many cases organisms become immune against certain injurious influences, as for instance the action of toxic substances, provided that these influences were active during a sufficiently long period; the immunity against disease-producing bacteria was especially regarded as a phenomenon of adaptation. These writers, therefore, explained immunity by including it among the larger class of adaptive phenomena. Although this attitude appears from a certain point of view as the most rational one, it may nevertheless in other respects be more fruitful to adopt the opposite attitude, which consists in trying to gain an insight into the mechanism of certain adaptive phenomena, by making use of the discoveries made in the course of investigations in immunity. This attitude seems also justified by the fact that a large number of instances of acquired immunity cannot be directly explained as adaptive phenomena. In these respects, therefore, the conception of immunity is wider than that of adaptation.[2] The main reason for taking the second attitude, however, lies in the fact that the phenomena of immunity have been partially at least accessible to an experimental analysis, much more so than other phenomena of adaptation.

The latter may be conveniently classified as internal and external, as adaptations of one part of the body to another part of the same organism and adaptations of an organism or of one of its constituent parts to the outer world, although these two kinds of adaptations must not necessarily be regarded as sharply distinct, inasmuch as it can be assumed that external conditions cause a primary internal reaction, which gives rise to a secondary internal change, the latter possessing the character of an adaptation and therefore representing directly an internal, indirectly however an external adaptation.

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[1] From the Pathological Laboratory of the University of Pennsylvania.

[2] T. H. Morgan in his book on "Evolution and Adaptation" includes the phenomena of immunity among the adaptive processes. He expresses, however, the view that certain of these phenomena could not be explained as due to any selective processes. The following remarks, the partly hypothetical character of which is quite apparent, were written with the aim of connecting a number of somewhat isolated facts, and of suggesting the possibility of an extension of such considerations to different fields of investigation.

It appears possible to connect certain characters, whose usefulness for the organism possessing them, seems to justify their classification among the adaptive phenomena with other facts which have been found by the experimental study of immunity. A few adaptations of this kind may be selected to demonstrate this connection. In the tissues of vertebrates and invertebrates, substances are present which have a strongly accelerating effect upon the coagulation of the blood. If a wound is made and the blood escapes, the contact with these tissues has a tendency to stop the bleeding and prevent the animal from bleeding to death. The tissues of each class of animals are especially adapted to the fibrinogen of their own blood. The blood of a bird coagulates more quickly under the influence of the tissue of a bird than of a dog or of a frog. The blood of a turtle coagulates more quickly under the influence of the tissues of a turtle than of the tissues of a mammal or of a bird. The same holds good of frogs' blood. Invertebrate blood, like that of a lobster, is not at all influenced by the tissues of vertebrates, but very powerfully so by the tissues of the lobster and to a less degree by the tissues of some other invertebrates. Here we have apparently to deal with a specific adaptation. A certain relationship exists between blood and tissues of one class of animals; a specific relationship, which proves very beneficial to the animal. A somewhat analogous fact is mentioned by Duclaux. He states that it is well known to manufacturers of dairy products that the milk of a certain species of animals is more rapidly coagulated by the rennin of the same than of another species.[1]

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[1] Although the analogy between these two facts is clear, the usefulness apparent in the case of the tissue coagulins, seems to be absent in the case of the specificity of rennin. The latter fact can therefore not be included among the phenomena of adaptation.

Certain parasitic animals, as the leech and anchylostoma, live entirely or partially by taking into their digestive system the blood of the host. In regard to anchylostoma it is not quite certain whether it sucks the blood of the host directly or whether it obtains the blood together with parts of the mucous membrane of the internal canal which it inhabits. Blood of the host certainly forms an important part of the food of the parasite. Both of these worms contain in the anterior part of their body, substances which strongly inhibit the coagulation of the blood. Thus the blood is kept in a liquid state and the sucking of the blood and probably also its digestion and resorption are rendered much easier. This was demonstrated in the case of the leech many years ago by Haycraft and recently also in the case of anchylostoma. Here again we have to deal with a process which to some degree is specific, inasmuch as the substance which has such a powerful action in inhibiting the coagulation of the blood of the host is absolutely without influence on the coagulation of invertebrate blood. And there are even certain indications tending to show that this substance does not act equally strongly on all vertebrate blood, inasmuch as Sabbatani found that a similar substance in another blood-sucking animal, ixodes ricinus, acts much more strongly on dog blood, the blood which is sucked by this tick, than on rabbits' blood. I myself found in one experiment made that the substance which is present in anchylostoma, inhibiting the coagulation of the blood of the dog, was powerless towards the blood of guinea pigs.[1]

The fact that leech extract is without effect on the blood of the lobster makes it furthermore more probable that it is also without power upon the blood of the leech itself.

Snake venom, a most powerful poison for many vertebrates, is almost harmless for snakes, themselves. This is as Phisalix found at least partially due to the presence of an antitoxin in the blood of the snakes. The blood of these animals may therefore contain toxin and antitoxin side by side. This immunity of snakes against their own poison is of great significance, otherwise an injury of their tongue or any other part of their body by the teeth of the animal would be fatal.

A toxin similar to snake venom is contained in the abdominal

[1] This experiment needs to be repeated.

segments of scorpions. The scorpions possess, as Metschnikoff describes, in their blood an antitoxin against scorpion venom, which is very poisonous for other insects and to vertebrates. Certain desert animals are frequently exposed to the bite of scorpions, and in accordance with this fact, it has been recently found that such animals are not susceptible to this poison, although other animals nearly related to the desert animals, but not living in localities where they are exposed to bites of scorpions, are easily poisoned. Whether the blood of these animals has any antitoxic action, does not yet seem to have been determined.

For a long time an explanation had been sought for the fact that the mucous membrane is not digested by the pepsin and hydrochloric acid, which are secreted by the cells of the mucous membrane. Recently Weinland has shown that the cells of the mucous membrane of the stomach contain a substance able to neutralize the action of the digestive ferment, an antipepsin. Although it is not certain or perhaps even unlikely that the presence of this substance is sufficient to account in itself for the power of resistance shown by the cells of the mucous membrane, yet it represents in all probability at least one important contributing factor and is therefore a cellular adaptation, without which life would be impossible.[1]

In the course of the study of the action of bacteria and their toxins on the animal organism, it has been found that injection of bacteria into the body of an animal may cause in the serum of the injected animal the appearance of substances, which produce an agglutination or even a solution of the bacteria (agglutinins, bacteriolysins). Injections of bacterial toxins may produce the appearance of antitoxin in the serum. Later it was found that this response of the animal organism was not limited to bacteria and their products but was also present in the case of injections of animal and plant cells in general and even of ferments and albuminous substances. The substances which after a certain period are to be found in the blood serum have all this in common that in some way they antagonize the organisms or substances, which had been injected and which had caused the appearance of the reactive substances. The way in which this antagonizing effect does take place is different in different cases.

[1] Metschnikoff, "L'immunité," Paris, 1901. Chapter XI.

Some facts are known in regard to the place of origin of such "antibodies" and in regard to the conditions which favor or inhibit their appearance. From a certain point of view we may at present perhaps distinguish three types of reactions.

1. If at certain intervals a solution of abrin is dropped into the conjunctival sac of an animal, substances are formed in the conjunctiva to which the abrin was applied, which are able to neutralize the injurious effect of abrin. In this case a local response of the cells on which the substance acted, has taken place.

2. If we inject substances, as cultures of the cholera vibrio subcutaneously, intraperitoneally or intravenously, antibodies are produced in organs far removed from the place of injection, as for instance in the spleen and in the bone-marrow. The mere injection of these substances is sufficient to produce this result.

3. There exists a third type of reaction, which seems to be of a more complicated character than the forementioned ones, and which hitherto has been regarded as a totally different process, but which seems to be essentially of a similar character. The normal pancreas, which is the source of several hydrolytic ferments usually does not produce lactase, a ferment capable of splitting the disaccharid lactose. If, however, an animal receives with its food a certain quantity of lactose, for instance in the form of milk, lactase is secreted by the pancreas. The introduction of a carbohydrate into the animal body causes, therefore, in this case the appearance of a specific substance able to destroy the carbohydrate. The lactase may be called an antibody. If we inject, however, lactose directly into the blood, lactase is not produced. The mechanism of the production of this substance seems to have been in the main cleared up by Bayliss and Starling and Bainbridge. It appears that through the introduction of lactose into the alimentary canal a substance is formed in the mucous membrane of the small intestine, which gradually passes into the circulation and causes the pancreas to secrete the lactase. It is possible to extract this substance from the intestines in vitro and to cause a secretion of lactase by injecting the extract into a vein. In this case it is not the original substance itself which by passing into the circulation causes directly the appearance of an antibody, but it is a second substance formed under

the influence of the lactose in the cells, with which the lactose or component parts of the lactose comes into contact.[1]

The difference between the second and third mode of action is not of such a character as to render it impossible to regard the latter as related to the former, especially as we do not know whether intermediary processes do not also take place even in those reactions, which are included under the second type. It is not impossible that a similar complicated character will be shown to exist in some reactions in the second class, which have been regarded as direct ones.

The presence of antibodies can, however, not always be demonstrated in cases of artificially produced immunity. It has for instance been impossible to obtain antibodies after injection of certain chemical substances, which are of a less complicated character than the albuminous substances mentioned above, *e. g.* certain alkaloids.

If we now turn again to the adaptations, which were mentioned above as occurring naturally, it is not difficult to see that a great similarity exists between the natural and the experimental reactions. The existence of an antipepsin in the mucous membrane of the stomach seems analogous to the production of antiabrin in the conjunctiva. In both cases we have to deal with an apparently local production of an antibody. If we find in scorpions and in snakes an antibody against their own venom circulating in their blood, it is not unlikely that we have to deal with a reaction of the second class. The fact of the leech and ankylostoma producing substances inhibiting the coagulation of the kind of blood they swallow may with some probability be classed among the reactions of the third type. The same probably holds good for the tissue coagulins. Whether in the case of the desert animals an antibody is present does not seem to have been determined ; it is, therefore, possible that we might have to deal with an acquired immunity without the presence of an antibody which can be demonstrated. On the other hand it is not unlikely that further investigation will show the existence of an antitoxin.

Plausible as the explanation given of some of these adaptations, as experiments of nature in immunity may appear, there seems to be some objection to applying it to cases in which the substance causing the immunization or adaptation is derived from the same animal in which the antibody is produced. Antibodies have been experimentally produced only in such cases in which certain substances derived from one animal were injected into another animal and usually those belonging to a different species. The experimental proof has, however, been given that in certain cases iso-antibodies can be produced, by the injection of animal cells or animal products into other animals of the same species. Furthermore it could be shown that such antibodies may act not only on the cells of another animal of the same species but even on the analogous cells of its own body. This for instance was found in the case of the spermotoxins. An antibody against its own spermatozoa can be produced in the same animal which has been injected with spermatozoa of another animal of the same species. Whether the spermatozoa are able to produce antibodies if they are injected into the same animal from which they are derived, has apparently not yet been investigated; but it appears not unlikely that it might succeed. Such an experiment gave negative results in the case of the blood cells which are however normally circulating in the blood. Certain other facts point to the conclusion that such autoantibodies may be formed without experimental interference under natural pathological and even normal conditions. If one kidney becomes chronically diseased by experimental interference so that tissue of the kidney is being resorbed, the serum seems to assume properties, injurious to kidney tissue of the same animal. If we inject such serum into the circulation of another animal of the same species, albuminuria appears as a sign that the kidneys have been injured. Even if this observation should be open to a different interpretation, there are other facts which suggest a similar conclusion. It is certain that such digestive ferments as trypsin pass normally into the circulation and may be excreted by the kidney. As might be expected from what has been said before, an antitrypsin exists in the serum, or the serum has antitryptic properties, just as the snake blood contains antitoxin against snake venon.

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[1] The deductions of Starling and Bainbridge have recently been controverted by Bierry.

Glaessner found even that the tryptic power of the serum varies at different periods of the day and that these variations indicate a certain relationship to the time at which the food is taken in and at which trypsin is secreted. This is very suggestive of the rapid formation of an antibody in response to the normal increase of a certain ferment in the circulation. In a similar way the blood serum of a horse and a pig was found by Briot, Roden and Korschun to contain normally an antibody against rennin. This natural antibody acts in the same way as the one experimentally produced by injecting rennin into other animals, probably combining with the rennin. A further observation strengthening the evidence that autoimmunizatory processes may take place is that during the puerperium an increase of isoagglutinins takes place in the human body. This is probably the result of the resorption of substances from the uterus and other organs. The resorption of these substances seems to modify the action of the blood serum on the blood cells of the same species of animals.

The specific character of the experimentally produced antibodies is quite marked ; these antibodies possess a certain relationship to that substance whose introduction into the animal organism caused the antibody to appear. This specificity is of a twofold character. In the first place the antibody reacts only against a chemical substance similar in composition to the one which caused it to originate, secondly there exists a species specificity. Substances apparently equally constituted, but derived from different species, behave differently towards the antibody, those substances showing the strongest reaction which are derived from the same species, or from a species similar to the one from which the substance was obtained whose introduction into an animal organism caused the formation of the antibody. The latter (and also the former) kind of specificity is not absolute, substances derived from nearly related species showing a similar although usually a weaker reaction.

Such a specificity may also be noticed in the adaptations described above. The snake and scorpion antitoxin are specific for snake and scorpion toxin respectively. The leech extract is without effect on the plasma of invertebrates, but acts only on the plasma of vertebrates ; in the case of ixodes and anchylostoma, the specificity may perhaps even go farther, so that the antiferment acts mainly on the blood of that species which is sucked by the parasite. In regard to the tissue coagulins, their specificity has already been mentioned above. We might be able to distinguish by the aid of this specificity for instance the muscle of a lobster from that of a blue crab. In the whole, however, we have to deal here rather with a class specificity than with a species specificity. The specificity is not so strongly pronounced in the case of the naturally produced antibodies as in those cases in which antibodies are produced artificially. Glaessner believes that a specificity of the antitrypsin of the blood exists even among different mammalians. The number of his experiments seems, however, to be very limited, so that at present this specificity cannot yet be accepted as proven. It is possible that also in this case a class specificity does exist rather than a species specificity. This may also apply to enterokinase, which activates trypsinogen. A species specificity does not seem to exist in this case, but a class specificity may nevertheless be present.

Both classes of substances, the experimentally produced, as well as the naturally occurring substances, are specific, not only in the sense that they are chemically different from substances found elsewhere but that they have a specific relationship to the substance which caused their appearance and that they indicate the species or class origin of this latter. Their action is, therefore, a selective one and they are not only specific in the sense of being chemically different from other substances but they are specifically adapted to a certain action on a very limited number of substances. We may, therefore, call these substances "specifically adapted" substances.

We suggested that the tissue coagulins owe their origin to a process of autoimmunization, the character of a certain fibrinogen determining the character of the coagulins of the same species or class. Tissue coagulins resemble very closely enzymes. The secretion of lactase, a typical ferment, is, as we saw, due to the introduction of lactose into the organism. This suggests that other ferments have a similar origin; they may be regarded as antibodies produced by a complex process of autoimmunization. The existence and interaction of ferments in the animal organism is perhaps the most perfect instance of internal adaptation.

So far we had only to deal with chemical not with structural adaptations. It seems, however, possible to extend the preceding considerations to structural adaptations. Ferments produce primarily chemical changes. But we know of chemical ferment actions which bring about structural changes in the medium in which they act. Thrombin in transforming fibrinogen into fibrin changes a colloidal fluid into a gelatinous more or less solid mass, which under the influence of pressure and traction may show a fibrillar structure not unlikely connective tissue. From a certain point of view, the fibrin ferment may, therefore, be regarded as a form producing ferment.[1] We might call it a morphogenic ferment. We have reason to assume that there exist other morphogenic ferments. So far mainly the splitting activity of ferments has been studied. It is not unlikely that the action of many enzymes may be reversible. There would then exist a large number of enzymatic actions, leading to the building up of complicated chemical compounds from relatively simple substances. Such an action would probably in part again be connected with the creation of definite structures, which would be different under the influence of different enzymes. These enzymes would have a specific species, and also a specific individual character and the analogous products created by such ferments would be different in different species and in different individuals.

Such an interaction of substances with the resulting formation of antibodies could especially be conceived of as taking place during embryonic development; it would tend to produce a correlation between different chemical and structural mechanisms which might in part at least account for the harmony which exists in the function and structure of different parts of an organism.

It has not yet been investigated as far as I am aware, whether some of the adaptations mentioned above have been acquired in each individual or whether they are hereditary. That might be doubtful, especially for instance in the case of the immunity of scorpions and snakes against their own venom. The latter alternative is, however, very likely in the case of the anticoagulative substance of the leech and of anchylostoma and in the case of the tissue coagulins. It might be of interest to examine the existence of such a substance in leeches which had never had an opportunity of sucking blood containing an active thrombin, *e. g.*, of leeches which had been nourished on blood which had been previously heated to 56°, and to investigate whether the desert animals raised at places where they are exempt from scorpion bites, would nevertheless develop immunity against scorpion bites.

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[1] The fact that the fibrin ferment produces organized substance has already been noted by Gautier.

If a hereditary fixation of the production of an antibody should take place, then such a substance could be formed independently of the presence of the substance which originally caused its production and the origin of such an adaptation would, if regarded as an isolated fact, not be apparent.

In connecting these different facts it was intended to show the analogy existing between several adaptations which are probably fixed by heredity and certain reactions of the animal organism which can be produced experimentally. If we shall be able to clear up more and more the phenomena of experimental immunization we may hope to explain by the same studies, one factor which seems to form an essential part of many adaptations.

Most of the reactions considered so far are useful for the organism in which the reaction is taking place. It is, however, possible to produce experimentally reactions of a similar character, in which no useful result can be recognized. It is furthermore not unlikely that the same principle (the formation of antibodies) may under certain naturally occurring circumstances lead to conditions which are injurious to the animal organism, as in the case of destructive processes taking place in one kidney. Uremia is explained by Ascoli through the formation of nephrolysins. It has been thought possible to explain on a similar basis through the formation of syncytiolysins conditions of such an acute pathological character as eclampsia. How far these views are correct, it is at present impossible to determine. The possibility must, however, be conceded that the same principle underlies equally very striking adaptations and somewhat less apparent disease producing processes. In recognizing this it will be easier to apply to the phenomena of adaptation the same causative investigation as to other phenomena.

THE REGENERATION OF A DOUBLE CHELA IN THE FIDDLER CRAB (GELASIMUS PUGILATOR) IN PLACE OF A NORMAL SINGLE ONE.[1]

CHARLES ZELENY.

The double chela described in the present paper was regenerated from the distal end of the stump of the smaller chela of a male fiddler crab (*Gelasimus pugilator*) after autotomy of that appendage at the breaking joint. The case seems of interest not only because it is a regeneration product but also because it belongs to the rare class of truly double appendages.

The crab which developed the double chela originally possessed a normal small chela on the right side and a normal large chela on the left and was operated on at Cold Spring Harbor on July 2, 1902.[2] The nerves of the two chelæ were injured by a needle, the injury in each case resulting in immediate autotomy of the appendage at the breaking joint. The crab was isolated and fed on *Mytilus* (horse-mussel). It moulted on August 4, 33 days after the operation. On both sides the regenerated chelæ were of considerable size and the right or smaller one was seen to be double. The crab was kept under observation for 17 days after the moult, or 50 days after the operation. Two views of the chela are given in the accompanying figure which shows the terminal four podomeres. The first,[3] second and third podomeres are of the typical single form. The fourth podomere, however, is divided terminally so that there are two entirely separate indices (*I*, *I'*). The split extends well down into the body of the propodite on this side. In connection with each index there is a dactylopodite (*D*, *D'*). The split on the dactyl side of the propodite, though not as deep as on the index side, nevertheless completely separates the two dactyls and forms a very distinct groove in the propodite.

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[1] "Contributions from the Zoölogical Laboratory of Indiana University," No. 67.

[2] The operation was performed as part of a series of experiments on compensatory regulation which are described in a paper now in press under the title of "Compensatory Regulation," *Journal of Experimental Zoölogy*, Vol. II., No. 1.

[3] Not shown in the figure.

One of the resultant pinchers (*DI*) the one more directly in the line of the axis of the chela is stouter than the other. In the stouter chela the dactyl and index are approximately of equal length. In the smaller chela (*D′I′*) the dactyl is very noticeably longer than the index. Except for these slight differences and the more slender character of the smaller chela it is a perfect mirror image of the other. As the split is deeper on the index than on the dactyl side, the dactylopodites are nearer together than are the indices. The planes of the two chelæ therefore converge slightly on the dactyl side and diverge on the index side. In life both parts of the double chela functioned as pinchers.

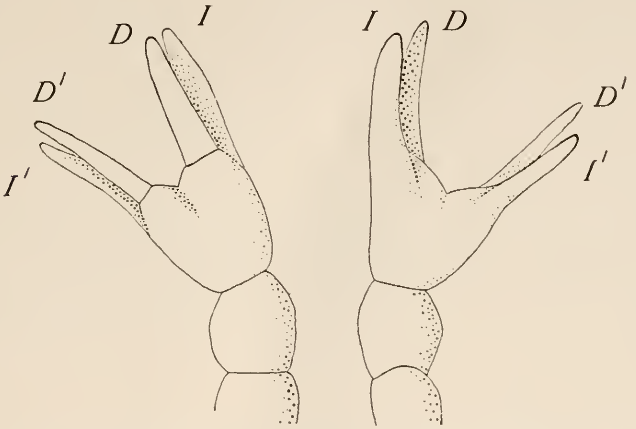

FIG. I. Double chela of *Gelasimus pugilator* (× 12). Left figure: viewed from pollex border. Right figure: viewed from index border.

DISCUSSION.

The double chela which has been described evidently comes under the group of true double appendages, a group the existence of which Bateson[1] was loath to admit since he considered the possibility that the supernumerary appendage in such cases is in reality itself double, as is true in a great number of the abnormal limbs so far described.

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[1] Bateson, "Materials for the Study of Variation," 1894.

The nearest approach to the condition of the present double chela that I have been able to find in the literature is the right cutting chela of a female lobster figured by Herrick on p. 147, plate E, of his "American lobster." This gives a chela which is apparently truly double from the carpopodite out. The two parts resemble each other very closely.

In Bateson's list[1] Nos. 832 to 835 come under the same head. Of these No. 833 (Fig. 200 I, p. 542) described by Faxon[2] as a right chela of *Homarus americanus* shows two dactylopodites articulating separately. The index is bifid at its tip and has two rows of teeth, one on each edge. If the separation of the dactylopodites had been slightly greater than shown in Faxon's figure and the split in the index had extended slightly further down on its side than the split in the dactyl a fair representation of the condition in the Gelasimus chela would have resulted. A similar modification in the degree of the split would bring Nos. 832, 834 and 835 into the same category.

There can be no doubt that the present regenerated double chela is a true double appendage, one part being a mirror image of the other except for the slight differences given above. The relation of the parts is such as one would expect if after the regenerating bud of the chela had become specified a mechanical force had partly separated it into two nearly equal parts by a quantitative split, *i. e.*, one passing in the plane of the future chela. With but a slight rearrangement of the materials in the two parts each is supposed to have been able to form a complete pincher in a manner entirely similar to that in the constriction experiments of Spemann and others[3] on Amphibian eggs by which double and partly double embryos were produced. The wider separation of the indices as compared with the dactyls is supposed to be due to the greater depth of the mechanical split on this side.

I have no suggestion to make as to the nature of the agency which caused the division of the embryonic mass. Spemann's experiments on the production of double embryos by constriction of Triton eggs by means of hairs and similar experiments of others as mentioned above, certainly indicate the possibility of producing double chelæ by similar methods used on the regenerating buds of Crustacean chelæ. Such experiments will be undertaken as soon as suitable material can be obtained.[1]

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[1] *L. c.*, pp. 541, 542.

[2] Faxon, *Bull. of the Mus. of Comp. Zoöl. Harv.*, Vol. VIII., No. 13, 1881.

[3] Spemann, Hans, "Entwickelungsphysiologische Studien am Triton-Ei," *Roux's Archiv*, Bd. XII., p. 224, 1901. Herlitzka, A., *Roux's Archiv*, Bd. II., pp. 352 ffl. Endres, H., *Roux's Archiv*, Bd. II., pp. 517 ffl.

If the explanation given turns out to be a true one it follows that the mass of embryonic cells which in the ordinary regenerating bud forms a single chela has because of the partial separation of its material been forced to develop a double one. Or stating the same facts in the convenient terms introduced by Driesch we may say that while the early regenerating bud has ordinarily a *prospective value* of a *single* chela the present case certainly shows that the same mass of cells has a *prospective potency* of *two* chelæ.

Indiana University,
February 8, 1905.

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[1] Andrews, E. A. ("An Aberrant Limb in a Cray-fish," Biol. Bull., Vol. VI., No. 2, January, '04) attempted to induce the development of monstrosities in the propodites of the chelate walking legs by making deep cuts in the adult limbs at the places where the new structures were expected to develop. Though 103 operations were made no positive results were obtained.

THE SPERMATOGENESIS OF SCUTIGERA FORCEPS.

GRACE MEDES.

Introduction.

In the present paper it is my purpose to note briefly some observations upon the spermatogenesis of *Scutigera forceps.* The spermatogenesis of *Chilopoda* has been the subject of a number of investigations during the last few years besides the earlier ones of Carnoy and Prenant. Carnoy, in his pioneer monograph on "Cytodiérèse Chez les Arthropodes," demonstrated the value of this material by a series of observations on *Geophilus*, *Lithobius*, *Scutigera* and *Scolopendra*, although many of his results have been disproved by later investigators. The most suggestive part of this work has to do with the various kinds of nucleoli and their composition and structure. He divided these bodies into four classes: *nucléoles nucléiniens*, *nucléoles plasmatiques*, *nucléoles mixtes* and *nucléoles noyaux*. In Chilopods, he found two of these: nucléoles noyaux in *Lithobius forficatus*, *Scutigera arachnoides* and *Geophilus;* and nucléoles plasmatiques in *Scolopendra dalmatica.*

Prenant worked upon *Scolopendra* and *Lithobius*, his results being very similar to those obtained later by P. and M. Bouin. These two, in connection with R. Collin, have worked upon several forms of Chilopods, *Lithobius forficatus*, *Geophilus linearis* and *Scolopendra morsitans.* These authors have concerned themselves principally with cytoplasmic structures, although they have given some attention to nuclear structures as well. The large nucleolar bodies found in *Lithobius* and *Geophilus*, according to them, are of an achromatic nature, and would correspond more closely to Carnoy's nucléole plasmatique. The chromosomes are not derived from this body, but arise from granules scattered through the nucleus.

The results obtained by Meves and von Korff upon *Lithobius forficatus* are, in general, in accordance with those of Bouin. They take the same view in respect to the origin of the chromosomes and regard in the same way the composition of the nucleoles.

Blackman ('01, '03) on the other hand, confined his work on *Scolopendra heros* principally to nuclear structures. According to him the large nucleolus found during the resting stage is almost entirely chromatic in nature, and from this body the chromosomes are directly derived at the opening of the active prophase. This structure, which he called the "karyosphere," would correspond to Carnoy's nucléole nucléinien.

TECHNIQUE.

The animal from which my material was obtained is the black-banded Centipede, or wall-sweep — *Scutigera forceps.* The specimens were collected during the spring and early summer. The animals were killed and their testes immediately removed and placed in Gilson's aceto-nitric sublimate, where they were left for a length of time ranging from twenty-four to forty-eight hours. They were then removed, washed in running water for several hours, run through ascending grades of alcohol from 30 per cent. to 70 per cent., where they were treated with iodine solution to remove the crystals of corrosive sublimate, and preserved in fresh 70 per cent. alcohol. The material was embedded by the usual method in paraffin ; sections varying in thickness from three to six micra were cut, fixed to the slide with albumen water and stained by one of the following methods :

The staining method productive of the most satisfactory results is Heidenhain's iron-hæmatoxylin. Sections were stained for observing different structures by varying the degree of extraction of the color. For some purposes, however, the sections stained with iron-hæmatoxylin and counter-stained with congo-red were more satisfactory. For the purpose of a micro-chemical test, Flemming's three-color method may be employed to great advantage. When stained in this manner, the different parts of the cell take on the following colors : the net-work stains a grayish purple, the centrosomes red, the chromatin in the dividing stages and in the karyosphere stains a transparent red, and in the diffuse condition found in the prophases and late telophase stains purple. The achromatic nature of the neucleoli is shown by the fact that they stain scarcely more densely than the linin and assume a similar grayish appearance.

Observations.

Early in the spermatogonial stages, cells may be seen aggregated in separate groups which are surrounded by definite membranes, or cyst-walls (Fig. 3). The number of cells in these groups varies considerably — in those counted, from eight to over a hundred. This may be due to either or both of two causes. According to the general conception of a cyst, it is an aggregation of cells consisting of all the descendants of one primary spermatogonium. Observations supporting this theory have been afforded by St. George, '76 (*Rana temporaria*), Henking, '91 (*Pyrrhocoris apterus*), Toyama, '94 (*Bombyx*), Montgomery, '98 (*Pentatoma*), Paulmier, '99 (*Anasa*), McGregor, '99 (*Amphiuma*), and Sutton, '00 (*Brachystola*). However, from observations upon later stages, it is evident that in *Scutigera* these cysts are not such permanent structures. By a series of careful counts taken during the spermatogonial and early spermatocytic stages, it has been found that the number of cells in cysts in the same stage of development exhibits considerable variation. The counts of early spermatocytes shows them to exist in groups of from twenty-eight to forty. In the spermatogonia the results were somewhat different. In these, the highest number of cells found in one cyst was one hundred and eight, while the average number in cysts supposed to contain cells in the last spermatogonial division was about sixty. This, to my mind, can be due to but one cause: The cysts are not definite structures, but either by fusion cysts containing a larger number of cells are formed, or, by the division of one, two cysts containing a reduced number are produced. Sutton, in his paper on *Brachystola*, has estimated that the number of cells in one cyst at the close of the division period is two hundred and fifty six. In this case, all of the descendants of one primary spermatogonium are contained within one cyst, but in *Scutigera*, either this is not the case, or the descendants of one of these primary spermatogonia are much fewer in number. That the former is true rather than the latter, is indicated by the fact that in those cysts containing the largest number of cells, all are in practically the same stage of development, and is further supported by the fact that, almost without exception, where cysts contain the smaller numbers, several composed of cells in practically the same stage of development are to be found in the same region of the testis.

The spermatogonia are small spherical cells, averaging about eleven or twelve micra in diameter. During the late prophase of the last spermatogonial division, the chromosomes lie in the clear nuclear area at the center of the cell (Fig. 1). These chromosomes, with the exception of one which stains more darkly than the others, present a dense granular appearance when stained with Heidenhain's iron-hæmatoxylin. The total number of these chromosomes is constantly thirty-seven (Fig. 1), which, if the accessory chromosome is excluded in both cases, is twice the number found in the spermatocytes, showing that this element remains distinct throughout the whole period of spermatogenesis.

During the metaphase of the last spermatogonial division the chromosomes may be seen lying in the equatorial plate, so placed as to form a ring; but owing to the minute size of the component elements and their close proximity to one another, they appear rather as a solid mass than as separate chromosomes (Fig. 2). When viewed in cross section, they present the appearance either of a plate densely crowded on the outside, with separate chromosomes scattered throughout the middle, or of a ring. The centrosomes may be seen lying in the cytoplasm at some distance from the cell-wall and at this period appear as minute particles staining an intense black with the iron-hæmatoxylin. Emerging from these points and directed towards the cell-wall are astral rays which are so fine and delicate as to appear mere elongations in the reticulum of the cytoplasm, rather than definite structures. The spindle-fibers, however, stain intensely and may be seen as separate threads connecting the chromosomes with the centrosomes.

In the early telophase, after the two groups of chromosomes have moved to opposite ends of the cell, the spindle threads, still staining intensely, lie stretched between these two masses, while the centrosomes have become lost (apparently) among the chromosomes and the astral rays have disappeared. As the telophase advances, the chromosomes become more granular, stain less deeply, and appear in the next stage (Fig. 3), as a mass of dense granular threads. Already the growth period has begun, and a slight increase in the size of the cell may be observed. At this period one other important feature may be mentioned. This has to do with that peculiar structure, first clearly recognized by McClung ('99) as a chromosome, and described by him under the name of accessory chromosome. This element may be distinguished from the other chromosomes at this period by certain peculiarities in its form and behavior. During the telophase, while the other chromosomes have been lengthening into diffuse, flaky segments, this structure has remained unaltered and still retains the stain with the tenacity of a chromosome during the metaphase. As is to be expected from its composition, its reaction to stains is quite different from that of the ordinary chromosomes. When stained by Flemming's three-color-method, this element takes the saffranin, whereas the others retain the gentian violet.

During the period immediately following, the cell gradually increases in size, and the reconstruction of the nuclear membrane takes place. By this time (Fig. 4), the cell has increased about one half and the nucleus is considerably larger in proportion than at any other period. The cytoplasm, moreover, stains more lightly and shows scarcely any structure. The chromosomes, immediately after assuming their thread-like shape, lie tangled together in a close heavy mass (Fig. 3), but by the time the nuclear membrane has formed, they begin to spread out through this vesicle and the separate threads may be distinguished (Fig. 4). While this is taking place, these threads of chromatin have gradually become more granular and diffuse, and very evidently fewer in number, while the small black body, above pointed out as the accessory chromosome, has apparently become larger. Into the composite structure, hereafter to be called the karyosphere (Blackman, '03), the diffuse masses of chromatin gradually accumulate (Figs. 5–6) until the nucleus outside of the karyosphere is entirely free of chromatin, and only the linin net-work is to be seen.

Another noteworthy feature characteristic of this period is the presence of metaplasm in variable amounts within the nucleus. Immediately after the reconstruction of the nuclear membrane, when the dense mass of chromatin is beginning to become more diffuse, so that the presence of foreign matter could easily be detected, the nucleus never contains more than the one small round body, the accessory chromosome, beside the mass of thread-like chromosomes. But as the cell increases in size, others gradually appear in the nucleus, and less frequently in the cytoplasm. These bodies are homogeneous, and, like the karyosphere, take a dense black stain with the iron hæmatoxylin. In number they vary widely, some cells being apparently entirely free of them, others containing them in large numbers; while in size they range from scarcely distinguishable particles to large spheres. These bodies seem to be largest and most numerous during the mid-growth period. As the prophase approaches, they seem to decrease in size, and by the time the karyosphere breaks up, their remnants appear as mere particles scattered through the nucleus. Those found in the cytoplasm persist about the same length of time, although there they are much less common than in the nucleus. These bodies present in both nucleus and cytoplasm, correspond to the "ergastoplasmiques" of P. and M. Bouin, which are said to arise through the breaking down of the spindle fibers and which disappear at the opening of the prophase. Meves and von Korff found similar structures in *Lithobius*, but these are often present throughout all the stages of mitosis.

While these changes have been in progress, the cell has gradually been enlarging and by the time of the opening of the active prophase, it is many times its original dimensions. Fig. 7, which represents a cell of about average size, has an approximate diameter of seventy-five micra, while occasionally one may attain the enormous size of one-hundred micra.

Throughout this period, the karyosphere has maintained about the same appearance, excepting its increase in size. During the earlier stages, at the time when the chromatin was accumulating, it appeared to be sharply granular, and to take the stain more densely in some regions than in others; but as the growth period progresses, the karyosphere, in more darkly stained sections, shows no differentiation of structure (Fig. 7). However, in sections two or three micra thick, from which the color has been sufficiently extracted, a careful study of the karyosphere under a high magnification, reveals the fact that it is not a homogeneous body, but is a complex mass, various parts of which react differently to the stain (Fig. 8). When colored with the iron hæmatoxylin, it appears to be composed of dark irregular masses, and between them smaller areas which stain brown and appear to be of a homogeneous consistency (Fig. 8, *b*, *c*, *d*). On the other hand, the larger masses are of slightly granular nature and commonly appear black. When the stain has been sufficiently extracted, however, they become grayish, and this color argues their chromatic nature, just as the brownish color without testifies to the achromatic nature of the karyosphere.

Evidences of vacuoles within the karyosphere may likewise be seen (Fig. 8, *b*, *c*, *d*) in thin sections lightly stained in the same manner, but their presence is shown much more clearly when in addition to the iron-hæmatoxylin congo-red is used (Fig. 8, *b*, *c*). These vacuoles in varying numbers may lie irregularly throughout the substance of the karyosphere.

From the growth period, the cell passes immediately into the active prophase. The first indication of this stage has to do with the appearance within the nucleus of the chromatic segments. As in *Scolopendra heros* (Blackman, '01), the first sign of activity is a very slight change in the structure of the karyosphere (Fig. 8, *e*). The texture of this body becomes more loose and the distinction between chromatin and achromatin becomes more marked (Fig. 8, *e*). This, however, is so slight that it would probably pass unnoticed were it not for the more striking phenomena which now follow. That the darker portions noted as occurring in the karyosphere at an earlier stage are chromosomes, appears very probable from the fact that they now seem to break off separately from its mass and to be directly transformed into short, thick, granular threads (Fig. 10).

It would naturally be supposed that, as the chromosomes leave, the karyosphere would become smaller, but such is not the case ; even after a number of chromosomes are to be seen lying in the nuclear area the karyosphere is apparently as large as before (Fig. 8, *g*, *h*, *i*, *j*). On close inspection, however, it may be seen that the vacuoles have greatly increased in size. Often these become united, and form one large vacuole at the center of the karyosphere, around which lie the remaining masses of chromatin (Fig. 8, *g*, *h*, *i*). When finally all these have emerged, there is left a mass of material staining more or less darkly, probably a true nucleolar portion of the karyosphere, and one small, round, dense black body, the accessory chromosome (Fig. 8, *i*). This latter presents exactly the same appearance as when last seen during the telophase, except for a slight increase in size, which may readily be referred to natural growth. The remaining mass, or true nucleolar portion of the karyosphere (fig. 8, *j*), now breaks up into small round bodies of more or less irregular size, which soon become indistinguishable from the remains of the metaplasm still lying in the nucleus during the growth period (Fig. 12, 13, 14). For this reason it is impossible to determine the ultimate fate of these bodies; for all seem to be of about the same size and appear to take the stain equally well.

Immediately after leaving the karyosphere, the chromosomes, as was shown above, shorten into dense granular cords (Fig. 9). These now undergo a longitudinal cleavage (Fig. 10), and the double thread of chromatin breaks up into a number of short portions of irregular size (Fig. 11). Whenever the position of these made counting possible, the uniform number of six in each half was to be observed.

Until the opening of the active prophase, the nucleus appears very clear, much more so than the cytoplasm, and contains a finely granular and irregular linin net-work, the meshes of which are much coarser than those of the cytoplasm. Metaplasm bodies left over from the preceding growth period may occasionally be seen caught in the meshes of this net-work, but with the exception of these and the karyosphere, no other structures are visible within the nucleus. Just preceding the emerging of the chromosomes from the karyosphere, however, the nuclear area becomes clouded over here and there with very thin, diffuse masses in which the segments of chromatin seems to become entangled as they pass out into the nucleus. It is these masses of achromatic matter which Bouin (*Lithobius forficatus*), Bouin and

Collin, '01 (*Geophilus linearis*), Meves and von Korff, '01 (*Lithobius forficatus*) probably mistook for chromatin. In addition to the fact that the chromosomes may actually be seen emerging from the karyosphere, the achromatic nature of this substance is clearly shown by its staining reaction.

At first the chromosomes appear to lie loosely in these achromatic masses (Fig. 8), but as the threads of chromatin split, becoming shorter and thicker, the enveloping substance likewise contracts, staining constantly a darker brown, until when the thread breaks into the smaller fragments (Fig. 11) this envelops them and appears to hold them together. Soon these balls of chromatin become massed more closely together in more or less irregular shapes (Fig. 11, 12), but they always assume as a general outline, the typical form of the tetrad (Fig. 12). This particular shape is often obscured by the unequal sizes of the chromatin fragments, and the distortion of the enveloping sheath which, now being less diffuse, takes a considerably darker stain (Fig. 13). But in all cases favorable for observation, *i. e.*, such instances as when the whole body appears lying flat in one plane, the general outline of the tetrad is plainly discernible.

Thus it seen that while the tetrads in *Scutigera* present characteristics peculiar to themselves, still they are of the same general type found in other arthropods. The only detailed description of tetrad formation in Myriapods has been furnished by Blackman ('03), in *Scolopendra*. According to his description this occurs through a longitudinal and a transverse cleavage in the chromosome segments. The longitudinal division occurs first, and is followed by a bending of the two halves of the segments at their centers, giving the first indications of the second cleavage. "The short processes thus produced elongate at the expense of the quadripartite segment until a cruciform figure is produced, the four arms of which are of about equal length." In *Scutigera*, this process is naturally much obscured by the breaking up of the chromosome segments into the unequal fragments; but when these have again become united by the contraction of the segment, the resulting figure closely resembles that in a similar stage in *Scolopendra*.

As the chromosomes continue to contract, the smaller globules composing them become fused closely together, and from now on the chromosome always appears as a single structure (Fig. 13 *et seq.*). The enveloping sheath likewise continues to draw more closely about it, until it is finally indistinguishable from its contents (Fig. 15). By the time the last chromosome emerges from the karyosphere, those issuing first have already begun to undergo this operation; but, since each assumes the tetrad form very quickly after making its appearance in the nuclear area, presently all seem to be in an equally advanced stage of development (Figs. 12, 13, 15).

The history of the centrosome is one of the most difficult questions in the study of spermatogenesis of *Scutigera forceps*, on account of the peculiar nature of the cytoplasm. In structure this consists of an exceedingly fine and regular net-work, staining brownish with the iron-hæmatoxylin. A further difficulty is due to the presence in the cytoplasm of small bodies, probably of the same nature as the larger bodies mentioned above. These particles are indistinguishable from the centrosomes, except when the latter may be indicated with certainty by the surrounding astral rays; but these rays offer no sure solution of the problem, for they are of an extremely delicate nature, and endure for only a brief period. Carnoy encountered the same difficulty in *Scutigera arachnoides*, of which he says: "Les figures caryocinétiques y sont plus déliées et moins demonstratives" than in *Lithobius forficatus*, which he has already described as being hard to study on account of its having such dense and opaque cytoplasm.

Because of these difficulties, the centrosomes cannot be distinguished with certainty until the opening of mitotic activity. In the early prophase (Fig. 12), a pair of minute granules may be observed, situated in the cytoplasm at each side of the nucleus, about one third of the distance between its membrane and the cell-wall. These lie in a transparent clear space from which astral rays of exceeding delicacy extend out for a short distance into the cytoplasm, where they gradually become lost in the net-work. During the breaking down of the karyosphere and the formation of tetrads, these centrosomes remain stationary in the cytoplasm, and the asters maintain a nearly uniform size and strength.

The changes in the achromatic part of the nucleus during the progress of the prophases are as considerable as those in the chromatin. During the growth period the contents of the nucleus, consist of the karyosphere, metaplasm granules and a coarse linin net-work. The interspaces of this net-work are large and clear, contrasting decidedly with the thick yellowish appearance of the cytoplasm (Fig. 7). The appearing within the nucleus of the cloud-like achromatic substance, which later forms envelopes for the chromosomes, has already been described (Fig. 9). During this process, the net-work may be seen unbroken between these darker masses; but soon after the chromosomes commence to form, the threads of this net-work begin to coalesce and the meshes gradually become larger and more irregular (Figs. 10, 11, 12).

Thus we have the general appearance of the cell at about the mid-prophase (Fig. 12). The nucleus occupies the central position, while on each side, well out from its membrane, lies a centrosome. Each of these consists of two dark granules situated close together and surrounded by a very delicate aster. Within the nucleus the threads of the net-work have coalesced considerably and now form large and very irregular meshes. Several small, dark masses, either metaplasm or nucleolar part of the karyosphere, may be seen at various places in the nucleus. The chromosomes have split longitudinally, broken into small round unequal segments which have drawn together, and assumed the typical form of the tetrad. Around these, the achromatic masses which serve as envelopes, have contracted until no longer discernible.

The late prophase, judging from the comparatively few cells to be found in this stage, endures but a short period. The chromosomes continue to contract and to increase in density and staining ability until they are reduced to but a small fraction of their former size (Fig. 14, 15) when they lose their granular nature and stain an even, intense black. Immediately after continued contraction, they lose also the tetrad form (Fig. 16), and assume the shape of the "diplosome" described by Bouin (*Lithobius forficatus* and *Geophilus lineatus*). At the breaking down of the karyosphere, the accessory chromosome follows the others into the nuclear area. At first it may be distinguished from them by its small, regular form and intense stain. But when the others have contracted into the dumb-bell shape, they are of uniform size with the accessory, stain similarly and thus are indistinguishable from it. But that this element assumes its position also in the equatorial area at the time of the metaphase is shown by the fact that there are nineteen chromosomes now present whereas but eighteen chromatin segments could be seen emerging from the karyosphere, and but eighteen tetrads observed later.

As the cell approaches the metaphase, the karyoplasm continues to break down, and assumes more and more the appearance of separate threads. At first these are wavy and irregular and often much branched, being, in fact, but segments of the linin reticulum. Later, however, at the time of the disappearance of the nuclear membrane, they straighten and lie like single threads in the cytoplasm. The centrosomes, meanwhile, have remained in the cytoplasm at some distance from the nuclear membrane. As this fades away, the threads formed from the network of the nucleus stretch from one centrosome to the other, and, losing their granular consistency, take on the form of distinct, definite fibers.

Preceding the breaking down of the nuclear membrane, the nucleus often wanders from its central position in the cell and assumes a position near the cell-wall. Bouin ('01), in *Geophilus linearis*, describes two methods of cell-division; one "division axiale," which represents the usual type where the centrosomes lie at opposite poles of the cell with the spindle between; the other, "division laterale," in which the spindle lies near the periphery and is often tangent to the cell-wall. In the latter case, he says: "Les corpuscules centraux et les sphères s'attachent contre la face interne de la membrane cellulaire, la ligne que les réunit est souvent d'une longueur moindre que le grand diametre de la cellule . . . Les extrémités du fuseau peuvent se trouver si voisine des centres cinétiques qu'elles paraissent se continuer avec la substance de ces formations."

But although cells answering to this description are often to be found in *Scutigera* before the metaphase, there does not seem to be two methods of division; for the more nearly the cell approaches the metaphase, the more nearly the spindle extends through its central axis.

The fibers of the spindle, when first formed, are short and the ends converge to a point in the centrosomes, which, at this period, do not lie upon the cell-wall, but retain their position in the cytoplasm (Fig. 16). However, the centrosomes gradually move apart and come to lie on the cell wall at opposite sides of the cell. Meanwhile, the chromosomes have taken up their position in the equatorial plate and the spindle fibers extend directly through the center of the cell. They do not now, however, extend from centrosome to centrosome, but lie free in the cytoplasm with ends toward these bodies. Their extremities, while converging somewhat, do not meet in a point. Blackman, '01 (*Scolopendra heros*), described a condition closely resembling this during the formation of the spindle. The centrosomes, however, are upon the nuclear membrane at the time it begins to disappear, but the formation of the spindle and its appearance in early stages closely resembles that found in *Scutigera*. As the centrosomes recede toward the cell-wall, the spindle fibers remain united at the same point (the "apical point") from which center of convergence parallel linin strands extend to the centrosome, while from around these latter emerge the astral rays. Meves and von Korff, '01 (*Lithobius forficatus*), describe conditions more closely resembling those found in *Scutigera* after the formation of the spindle is established, although during this process, the similarity is not so close. Before the disappearance of the nuclear membrane, the centrosomes, surrounded by the centrosphere, have already taken their place upon the cell-wall. During the metaphase, he describes them thus: "Die Spindelfasern liegen in einer hellen Substanz (wahrscheinlich Kernsaft) eingebettet. Nach den Polen zu konvergieren sie etwas; ihre Enden sind aber nicht mit einander vereinigt, sondern hören frei auf; diejeniger Strahlen um die beiden Central-körperpaare welche direkt auf die Enden der Spindelfasern zu verlaufen, treten mit diesen allem Anschein nach nicht in Kontinuität."

This mutual independence of the spindle-fibers and the astral rays in *Scutigera* is the more clearly shown by the great dissimilarity in their structure and general appearance ; for while the latter are very fine and delicate and do not appear to be separate threads, but resemble rather mere elongations in the network of the cytoplasm, the former are very heavy, stain an intense black, and present the appearance of thick wires, the ends of which may be distinctly seen lying free in the cytoplasm.

At the opening of the metaphase (Fig. 17), the two centrosomes lie at opposite poles of the cell, surrounded by the clear transparent area and the radiating astral rays. The spindle lies directly through the center of the cell, pointing toward the centrosomes but not extending to them. It consists of heavy, intensely staining fibers. At their extremities these converge, but do not unite. In the equatorial plate are the chromosomes, nineteen in number, not lying in a circle on the periphery of the spindle, but apparently scattered more or less irregularly in one plane through the equatorial plate. Division and separation of the chromosomes occur immediately, and the two groups soon come to lie at opposite ends of the spindle fibers.

As has been stated above, after the other chromatin has entirely left the karyosphere, the accessory chromosome separates from the nucleolar portion, which immediately breaks up into numerous small particles. When the nuclear membrane has disappeared, these particles are, of course, cast out into the cytoplasm. They are not dispersed at once throughout the whole cytoplasm, but in the metaphase, take up a fairly definite position at each pole of the spindle proper, midway between centrosome and equatorial plate. Thus, before the attractive force of the centrosomes has succeeded in pulling apart the chromosomes, these particles already lie in the cytoplasm beyond the extremities of the spindle fibers. Here they remain as long as the centrosome is discernible, but after the latter disappears they wander out throughout the cytoplasm and soon degenerate.

Each centrosome, from the time it first becomes visible, consists of two minute granules, surrounded by a transparent space from which the short and delicate astral rays emerge (Figs. 12, 17). But at this period, shortly following the separation of the chromosomes, each centrosome becomes surrounded by a small, spherical-shaped region (Figs. 18, 19), which, when the cells are stained but lightly by the iron-hæmatoxylin, presents a grayish appearance. In the center of this space, side by side, lie two black granules. When the cell is stained a longer period with the hæmatoxylin, this mass becomes very dark like the centrosomes, and shows an irregular outline, appearing as if formed by the fusing of the converging astral fibers. P. Bouin ('03), in a recent paper under the title of "Centrosome et Centriole" calls this entire structure, consisting of the two granules and the surrounding sphere, the centrosome, and each individual granule, a centriole; using this method to prove that the centrosome is not a permanent organ of the cell. But he seems to have misunderstood the meaning which Boveri, the originator of these terms, applied to them. The centrosome, as described by the latter, is "Ein Körper, an den die Sphärenradien direkt herantreten, ist das Centrosome." He tells us that it is present at all stages of cell-division, and divides to form the centers of the daughter-cells. According to his description this may, in the course of development, enlarge gradually, and become more complex, until just preceding the metaphase it may take on the form of a rather large, well-defined sphere in the center of which one or more minute central granules or centrioles may appear. Later, he adds as a test for distinguishing this enlarged centrosome from the centrosphere, that through the latter *astral rays may be traced*, while the former shows no such differentiation. Bouin accepts Boveri's definition for the enlarged centrosome during the metaphase, but rejects his definition of a centrosome during the prophase and declares the two granules occurring at that time to be the centrioles. In this manner he tries to prove that the centrosome is not a permanent organ of the cell. That his assertion is not valid in the case of *Scutigera* is proved absolutely by the fact that at no time during the existence of the sphere surrounding the two granules does it appear sharply defined from the astral rays, but these seem to traverse it and radiate from the two central granules which are invariably discernible in those cells not too darkly stained.

After the chromosomes have reached their destinations at the extremities of the spindle-fibers (Fig. 19), the asters surrounding the centrosomes fade away and the centrosomes themselves disappear. Either they lose their staining capacity or, having no surrounding rays to mark them, are indistinguishable from the numerous particles, the remains of the nucleolar portion of the karyosphere, massed at this point. The chromosomes, having reached the end of the spindle-fibers at first appear to be attached to them at their extremities (Fig. 19), but as the fibers at once begin to undergo degeneration and to become considerably shortened, the chromosomes soon lie free in the cytoplasm (Fig. 20). While attached to the fibers they lie massed together (Fig. 20), but afterwards they spread over a greater area (Fig. 21) and the separate elements may be seen. Each daughter-cell contains eighteen chromosomes, which are still dumb-bell shaped, but slightly granular and less intensely staining than before. One cell contains in addition the accessory chromosome (Fig. 21), still homogeneous in consistency and staining black. This element, on account of its resemblance to the other chromosomes (during the metaphase) could not be distinguished from them, but at this period may plainly be discerned as it retains the same form and staining capacity possessed throughout the metaphase.

During the period immediately following the reconstruction of the nuclear membrane takes place. This process is the most remarkable phenomenon in the spermatogenesis of *Scutigera*, but as it takes place much more slowly in the second mitosis, affording better occasion for the study of the separate steps, it will be discussed in connection with that division.

The period between the two divisions passes quickly, although the chromosomes undergo great changes in appearance. During the metaphase of the first division, as was stated above, they assume a dumb-bell shape and appear homogeneous (Fig. 17). Immediately after this division they again present the same form and appearance, although reduced in size. As the telophase approaches, they become somewhat more granular, but retain the same general outline. After the reconstruction of the nucleus has taken place, however, the chromosomes gradually become more and more granular until they lie spread through the nuclear area as diffuse masses. Their identity is not lost, although they retain no definite form. As the membrane again breaks down preparatory to the second division, they gradually contract and assume once more their dumb-bell shape.

The formation and appearance of the spindle fibers up to the time of the metaphase has been described above (Fig. 16). During this period they lie like heavy black threads in the transparent substance derived from the nucleus (Fig. 17), one fiber being attached to each chromosome and so distinct that it may be traced from one end of the spindle to the other. The net work throughout the cell, except in the immediate vicinity of the centrosomes, is of the same regular nature characteristic of the cell during the resting stage, and shows the same density marking it throughout the prophase. Around the centrosomes, however, the astral rays are beginning to elongate and strengthen. At the same time the net-work surrounding the spindle becomes more ragged, the cytoplasm through the equatorial plane lighter and freer from linin until by the time the chromosomes have reached their destination at the extremities of the spindle fibers (Fig. 19) the entire area in the vicinity of the spindle is clearer, and scarcely any trace of the net-work can be discerned. Gradually now the spindle fibers spread across the equatorial plane (Fig. 20). As those upon the outside approach the periphery of the cell, where the constriction of the wall has already commenced, they become shorter and weaker as their extremities become dissolved in the cytoplasm through which they are scattered. At no time are the astral rays strong nor do they ever consist of heavy distinct fibers like those of the spindle, but they appear much lighter and at the extremities become lost in the net-work of the cytoplasm. They extend between the centrosomes and the equatorial plate but they never cross in this region. Indeed, scarcely ever can they be distinguished this far, but fade into the network of the cytoplasm about half way from the centrosomes to the equatorial plane (Fig. 17). However, the whole cytoplasm of the cell, extending from the centrosomes at the poles to the constricting cell-wall at the sides, soon becomes transparent and entirely free from net-work, apparently indicating some influence of the centrosomes on this area (Fig. 20).

As the constriction of the cell-wall continues, the regular network of the cytoplasm begins to re-form about the periphery, and the spindle fibers lying in the equatorial region become pushed together until they form a sheaf-shaped bundle, the ends of which extend far into the cells. The fibers are exceedingly numerous, but not so heavy nor so darkly staining as when attached to the chromosomes during the metaphase and directly following it. Considering the size of the spindle, its disintegration occurs in a remarkably short time, indeed it begins as soon as the chromosomes have reached the extremity of the spindle. Until this time, the fibers were extremely heavy and presented a smooth appearance. But now they gradually become fainter and more granular, while those at the sides appear more and more feeble, until they can scarcely be distinguished from the network of the cytoplasm (Fig. 20). By the time they are pushed in to form the sheaf-like bundle (Fig. 24), the separate threads are of extreme fineness and delicacy. As the cell-wall presses inward, drawing the spindle together, a row of darkly staining bodies forms on the fibers in the equatorial plane, and appears to fuse. Only those fibers on the periphery seem to be affected thus, so that these granules do not form a plate, but a band around the circumference of the spindle. The period between the formation of the sheaf and the disappearance of the fibers passes quickly, although there is a wide variation in the time at which this takes place. In some cases the chromosomes are still in the vesicular stage after the cells have separated and the spindle fibers become greatly reduced in strength and numbers (Fig. 22), whereas in other instances (Fig. 24), a complete reconstruction of the nucleus has taken place before invagination of the cell-wall is completed. However, in no case does the second division commence until the spindle has so completely broken down that no trace of it remains.

During this entire period, the centrosomes are not discernible, but in the next metaphase they have reappeared, one granule on the cell-wall at each pole, surrounded as in previous division by a centrosphere and radiating fibers. These present an appearance almost identical with those in the preceding mitotic figure and bear about the same relative strength to the spindle fibers. As the nuclear membrane breaks down previous to the second mitotic division, the chromosomes all resume the same dumbbell shape as in the telophase of the former division. The accessory chromosome again becomes lost among the others as they now possess the same form and staining reaction that it has maintained thus far throughout its course.

The metaphase passes rapidly, and soon the chromosomes, as in the preceding division, come to lie at the extremities of the spindle, which also closely resembles that of the first spermatocyte. The chromosomes soon become detached from the ends of these fibers and lie free in the cytoplasm. Generally they are aggregated more or less closely in one region, near where the extremity of the spindle fibers lay, but often they become scattered irregularly through the central area of the cell.

And now an occurrence takes place which has not been previously described in Myriapods, nor am I acquainted with the phenomenon in the male cell of any form, although it is a common occurrence in the egg cell. Exception to this statement should perhaps be made regarding somewhat similar conditions reported by Meves in *Paludina* and *Pygæra.* In this work Meves ('03) reports a vesicular condition of the chromosomes in the telophase of both spermatocyte mitoses, but this may persist after the nuclear membrane is formed. The conditions in *Scutigera* are therefore more comparable to those manifested by various ova. There is no immediate formation of a nuclear-membrane, but each separate chromosome, as it disintegrates, becomes enclosed in a membrane of its own, thus forming a structure similar to a nucleus, but containing only a single chromosome (Fig. 25). The diameter of each of these is no greater than twice that of the chromosome itself. During the formation of these vescicles, the chromosomes become somewhat granular and often lose entirely their typical dumb-bell outline. They assume various arrangements in different cells; sometimes they lie close together, often they are scattered throughout the central region of the cell. Immediately after forming, however, these vesicles begin to fuse with one another, gradually producing large vesicles which contain more chromosomes. There seems to be no definite order of union, for sometimes they join in pairs (Fig. 26) and these gradually come together, while at other times several unite, forming a large vesicle around which are clustered smaller ones (Fig. 27). This latter case seems to occur more frequently although the former is often met with.

As was suggested above, this process occurs in both spermatocyte divisions, but in the first, it takes place much more quickly than in the second, and thus offers fewer examples for study. In case of the first division (Fig. 22), these vesicles always lie close against one another, whereas at the close of the second division, they are often widely scattered through the cytoplasm (Fig. 25). In the first division, too, the nucleus immediately after fusing becomes spherical, and all traces of its vesicular condition are lost. But at the close of the second division, the nucleus, in place of at once becoming spherical, retains for a considerable period traces of its former condition (Fig. 28), and often loses them only when transformation into the spermatozoön takes place. The membrane surrounding the nucleus at first appears very thin, like that of the vesicles; but soon after fusion is completed, it seems to increase considerably in thickness, and resembles that of the first prophase.

The only case which I have found of an occurrence resembling this was reported by Sutton, '00 (*Brachystola magna*). The metaphase is passed without exhibiting any unusual phenonenon. But during the telophase, an occurrence somewhat similar to that found in *Scutigera* takes place. "Each chromosome, on reaching the pole, begins to disintegrate, and at the same time, reconstructs its share of the nuclear membrane as a closed vesicle about itself. Later, all these vesicles become intercommunicating at their polar extremities, with the exception of one, which remains absolutely independent throughout its entire existence. The chromatin of the ordinary chromosome becomes diffused evenly in the nuclear space, while that of the one in the separate vesicle (the accessory chromosome) is deposited upon the inner surface of its capsule."

The spindle of the second mitotic division is markedly different from that of the first, although in the metaphase the two bear a distinct similarity. During the telophase, the breaking down of the net-work of the cell in the equatorial region and the spreading of the spindle fibers across this area is essentially the same as in the previous division, except that the fibers here are considerably fewer. As a result, no prominent sheaf-like bundle is formed when the constricting of the wall occurs, but these fibers lie like a few strands obliquely through the cytoplasm (Fig. 25, *et seq.*). As soon as the chromosomes reach their destination at the extremity of the spindle, the net-work of the cell commences to re-form, and, within a remarkably short period, all trace of division is lost from the cytoplasm.

Soon after the second division occurs, the centrosomes also disappear. In the case of the former division, it was stated that they either lost their staining capacity or became indistinguishable from the remains of the karyosphere massed in their vicinity. But in this instance, the former is shown conclusively to be the case, partly because of the fact that here there are no such granules with which they could become confused, and partly for the reason that for a considerable time the aster remains visible, although at the point from which the fibers radiate, no centrosome is to be seen (Figs. 25, 26).

I wish to express my gratitude to Dr. C. E. McClung under whose direction the work was done, and to Mr. M. W. Blackman, for assistance in supplying material and for many valuable suggestions.

Laboratory of Zoölogy, University of Kansas,
June 8, 1904.

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XPLANATION OF PLATES.

All drawings are from camera-lucida outlines, taken with a B. and L. one-twelfth inch oil immersion objective and one-half, three-fourth, 1 inch B. and L. oculars. In reproduction a reduction of one-sixth has taken place.

EXPLANATION OF PLATE II

FIG. 1. × 2,000. Spermatogonium in late prophase, about the time of the breaking down of the nuclear-membrane. The chromosomes, 37 in number, are spread over the central region of the cell. All are of a slightly granular consistency, except one, the accessory chromosome, which is homogeneous.

FIG. 2. × 1,000. Division of the last spermatogonium. (*a*) Chromosomes arranged on periphery of spindle or (*b*) scattered across equatorial plate. (*c*) Division figures typical. Centrosomes at apices of spindle. (*d* and *c*) Early and late anaphases.

FIG. 3. × 1,000. Late telophase of last spermatogonium. All chromosomes except accessory have become granular and lengthened into threads. The accessory is still homogeneous.

FIG. 4. × 1,000. Early spermatocyte. Chromatin segments beginning to form the karyosphere. The remaining segments scattered over nuclear area, in the form of long, granular threads. Growth period commenced.

FIG. 5. × 1,000. Later stage. Karyosphere becoming larger. Remaining segments of chromatin spread over the nuclear area in the form of very diffuse masses.

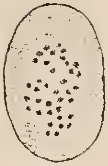
FIG. 6. × 1,000. Chromatin almost all in the karyosphere. Metaplasm bodies spread through the nucleus in considerable quantities. A less amount in the cytoplasm.

FIG. 7. × 1,000. Cell of mature size ready for the prophase. Karyosphere shows no differentiation. Metaplasm bodies decreased in amount. Network in cytoplasm is finer and stains more densely than that of nucleus.

FIG. 8. × 1,500. Karyosphere as seen in various stages. (*a*) During early growth period. (*b*) Just preceding the active prophase. Shows slight differentiation. Several vacuoles present in the matrix of spongy chromatin. (*c*) Karyosphere of same stage, showing the chromatin massed in more definite areas. (*d*, *e*) Very early prophases. Chromatin has become less densely massed; vacuolar substance has increased in amount; and has aggregated into one or several large vacuoles. (*f*) Part of chromatin has left the karyosphere. (*g*) Later stage. But little chromatin remains within the karyosphere, and this is massed about the large central vacuole. (*i*) All chromatin has left the karyosphere except one chromosome, which is probably the accessory. (*j*, *k*) Stages in the disintegration of the karyosphere, after the chromatin has all emerged.

FIG. 9. × 1,500. Early prophase. Chromosomes just commencing to leave the karyosphere. Cloud-like masses of achromatic matter beginning to arise in the nuclear area.

FIG. 10. × 1,500. Later stage in prophase. Chromatin segments granular and in the form of split threads.



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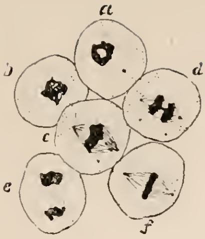

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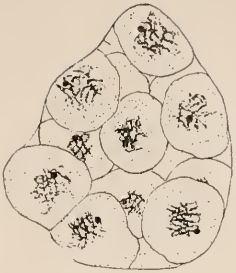

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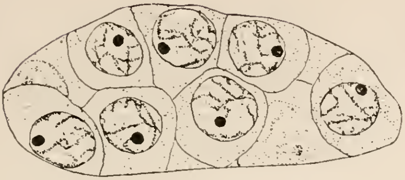

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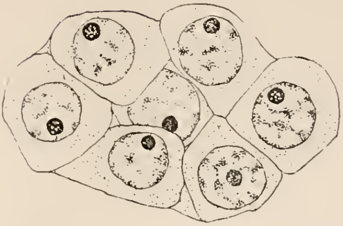

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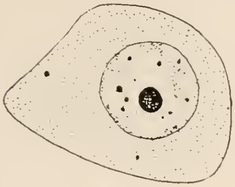

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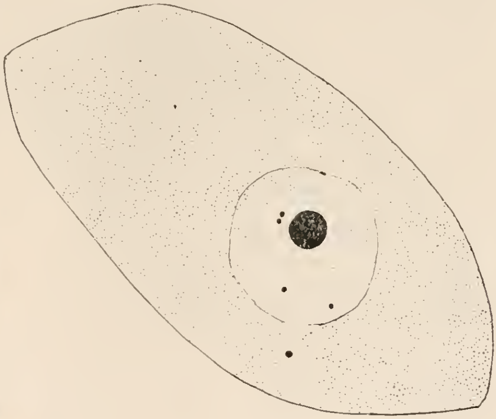

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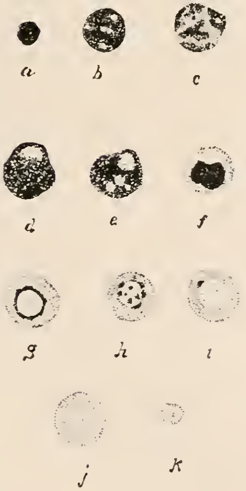
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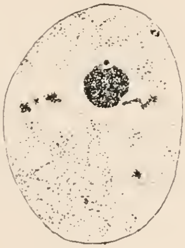

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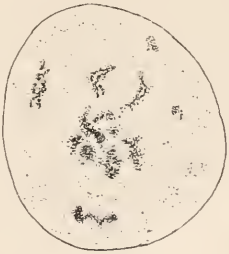

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EXPLANATION OF PLATE III

FIG. 11. × 1,500. Later stage. Chromatin segments have broken into a number of small fragments. These are beginning to contract to form tetrads.

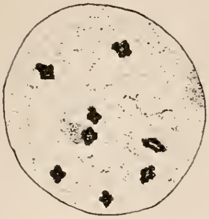
FIG. 12. × 1,500. Entire cell during mid-prophase. Chromatin in the form of cross-shaped tetrads. Centrosomes in the cytoplasm midway between nuclear membrane and the cell-wall.

FIG. 13. × 1,500. Later stage. Tetrads more contracted. Linin net work beginning to break down into more definite, less branched fibers.

FIG. 14. × 1,500. About the same stage. Tetrads in various stages of advancement.

FIG. 15. × 1,500. Later stage, shortly preceding the breaking down of the nuclear membrane. Chromosomes are nearly all homogeneous, but still preserve their tetrad outline. Nucleolar part of karyosphere still intact.

FIG. 16. × 1,000. Very late prophase. Nuclear membrane has disappeared and spindle is forming. Chromosomes have changed from tetrad to dumb-bell shape. Nucleolar remains of karyosphere grouped irregularly in the cytoplasm about the centrosomes.



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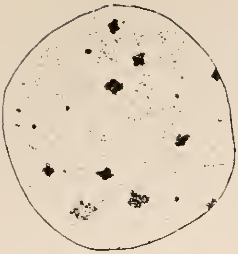

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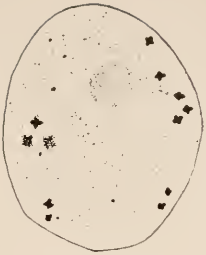

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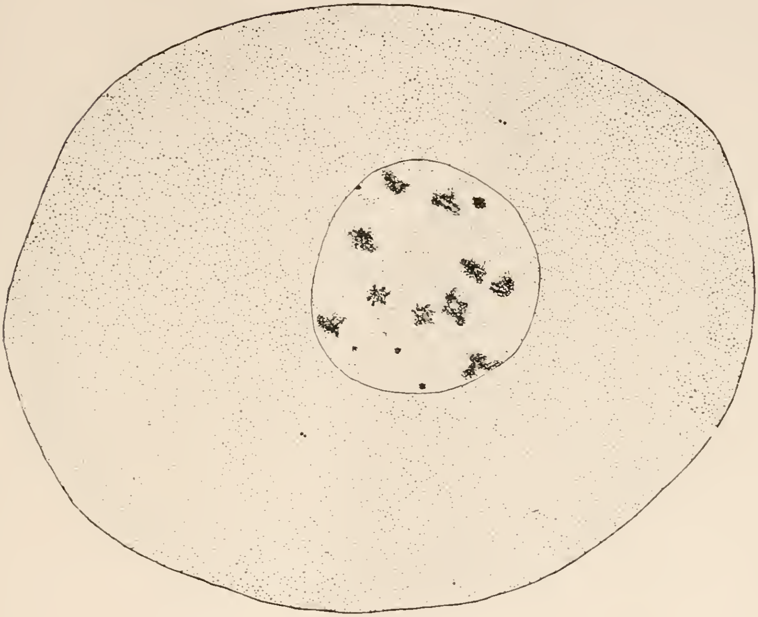

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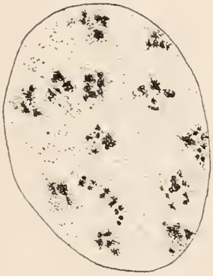

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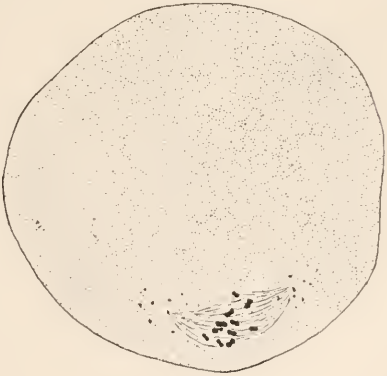

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Explanation of Plate IV

Fig. 17. × 1,000. Metaphase of first spermatocyte. Chromosomes grouped in equatorial plate. Centrosomes on cell-membrane. Spindle fibers do not extend to centrosomes, but lie free in the cytoplasm, their ends converging slightly. Nucleolar remains of karyosphere drawn to extremities of spindle fibers.

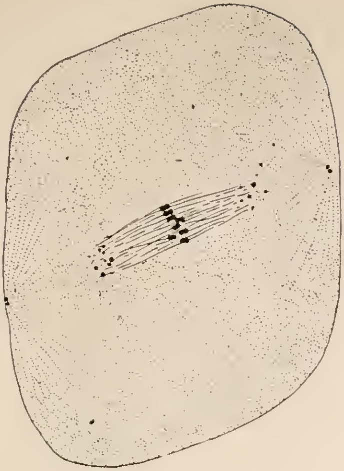
Fig. 18. × 1,000. Early anaphase. Chromosomes have divided, and again assumed their dumb-bell shape. Nucleolar remains of karyosphere drawn farther toward the centrosomes. Net-work still regular.

Fig. 19. × 1,000. Late anaphase. Chromosomes at poles of the spindle. Nucleolar remains of karyosphere drawn almost to cell-wall. Net-work beginning to break down in equatorial plate.

Fig. 20. × 1,000. Early telophase. Chromosomes massed at ends of spindle fibers, which have spread across equatorial region and begun to disintegrate at the extremities. Centrosomes have disappeared. Region from centrosomes to equatorial area considerably lighter, showing influence of centrosomes.

Fig. 21. × 1,000. Spindle forming into sheaf-shaped bundle. Chromosomes somewhat spread, becoming granular — except accessory, which is still homogeneous.

Fig. 22. × 1,000. Telophase of first spermatocyte. The vesicles in each one of which a chromosome has become enclosed are beginning to fuse.



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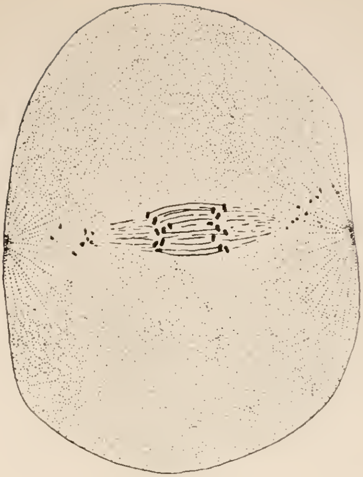

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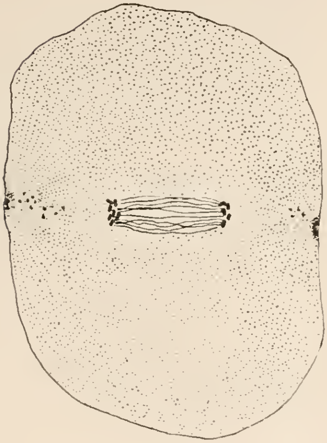

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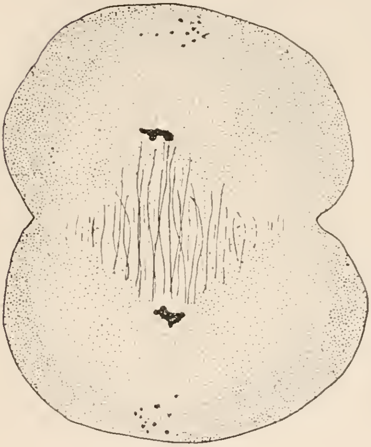

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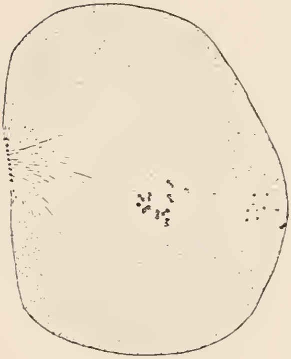

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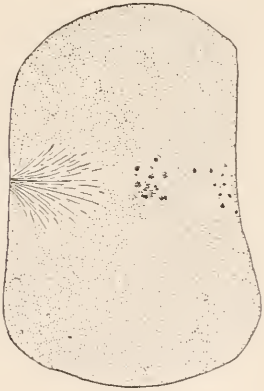

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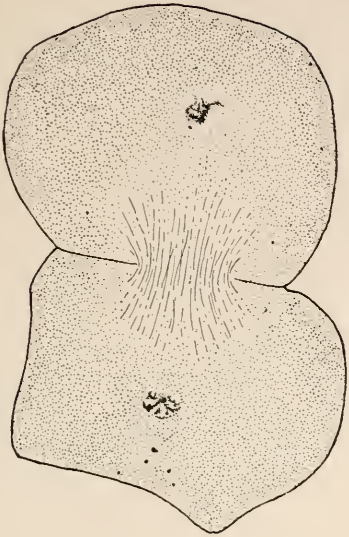
EXPLANATION OF PLATE V

FIG. 23. × 1,000. Telophase. Nucleus just forming. Showing the slowness of the forming of the cell-wall in some instances. Compare with Fig. 22.

FIG. 24. × 1,000. Late telophase. Nuclear membrane formed. Chromosomes become diffused. Cell "A" shows accessory chromosome, which is absent in "B."

FIG. 25. × 1,000. Telophase of second spermatocyte. Each chromosome is enclosed in its separate vesicle. Centrosome has disappeared, but astral rays are still present. Spindle fibers persist as a few strands lying in the cytoplasm.

FIG. 26. × 1,000. Slightly later stage. Chromosome vesicles have begun to fuse.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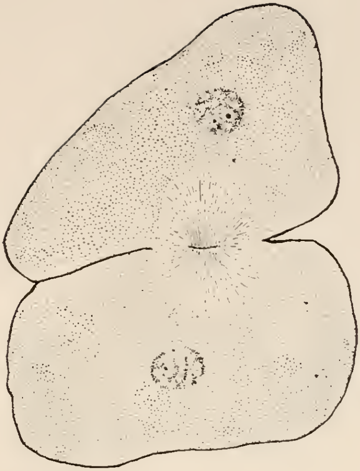
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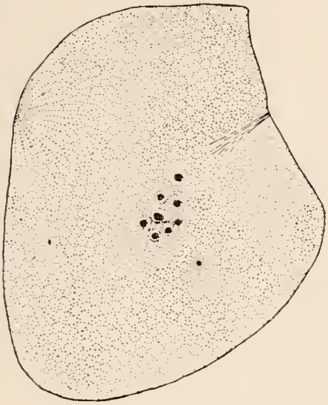
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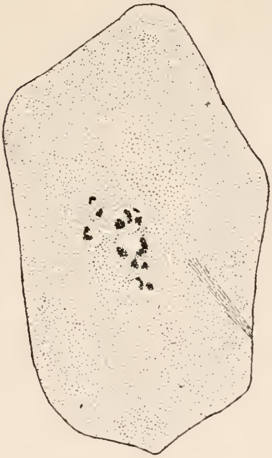
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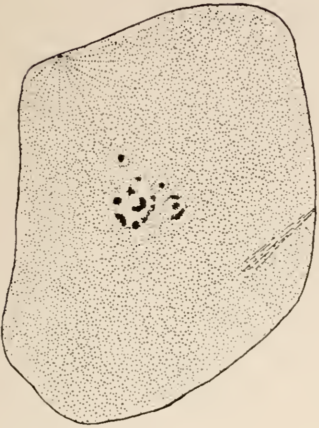
Explanation of Plate VI

Fig. 27. × 1,000. Still later stage. Fusion of chromosome-vesicles more advanced. Several of these have united, leaving part of the chromosomes still in separate vesicles.

Fig. 28. × 1,000. Fusion of the chromosome-vesicles complete, but the nucleus still retains a slight trace of its former vesicular condition. Nuclei of this shape are often to be found until transformation into spermatozoa takes place. All trace of astral rays has disappeared.

Fig. 29. × 1,000. All trace of vesicular stage has disappeared from the nucleus. Typical cell at the close of the second spermatocyte division.



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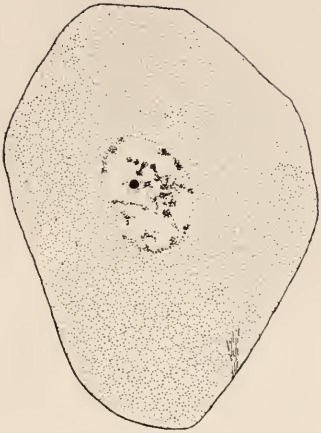

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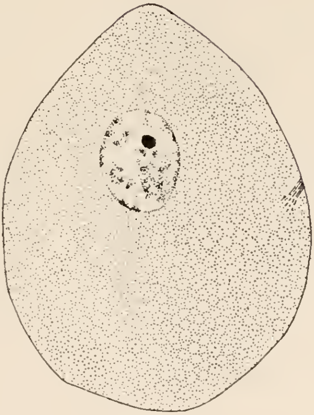

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INCOMPLETE ANTERIOR REGENERATION IN THE ABSENCE OF THE BRAIN IN LEPTOPLANA LITTORALIS.

LILIAN V. MORGAN.

Previous experiments on the regeneration of planarians, more particularly of marine forms, seem to indicate that the presence of the cephalic ganglia is an important factor in regeneration. The following experiments were carried out to test the question further. The species used was a large marine planarian, *Leptoplana littoralis*, very abundant at Pacific Grove, Cal., found under wet stones just above the low-water mark.

Lillie[1] and Morgan[2] have observed in *Dendrocœlum*, that the power of regeneration is limited in posterior pieces, the regeneration anteriorly never being complete if more than about the anterior third of the worm is cut off. Schultz,[3] found in *Leptoplana* that anterior regeneration of a posterior piece cut at any level posterior to the cephalic ganglia is very slow and rarely if ever complete. Child[4] observed the same fact, and also in the case of lateral regeneration that "when they [the cephalic ganglia] are absent neither they nor the lateral part of the head removed are regenerated, though lateral regeneration of other parts may be more or less complete in the absence of the ganglia." In *L. littoralis* there is also a very marked difference in the rate and amount of regeneration of different kinds of pieces, and also in the behavior of the pieces.

Pieces of any size and shape, in which the cephalic ganglia remained, responded quickly to stimuli that affect the normal worm, and in general behaved like normal animals; moreover those that lived regenerated rapidly. Even small pieces (cut out of the head with a straw), containing the eye-spots and cephalic ganglia and very little tissue besides, responded readily to light and touch, and crawled actively ; in all cases in which such pieces survived, they quickly regenerated tissue in every direction, and in less than two weeks presented the appearance of small worms with heads very large in proportion to their tails (Fig. A, 7, 8). Pieces without the cephalic ganglia, on the other hand, behaved differently. They were sluggish in their movements, responded slowly and did not even stay under water, but were often found dried up on the sides of the dish. Also, as Child and Schultz found in the forms which they studied, anterior regeneration took place only to a very limited extent. Pieces cut at any level posterior to the ganglia often healed in such a way as to apparently prevent the growth of new tissue. After the worm was cut, the sides stretched very much and actually met in front of the worm, bringing the two halves of the cut surface in contact, so that they grew together, the point where they met in the middle line being raised above the level of the surface on which the worm crawled. To avoid such a closure of the wound, worms were in some cases cut so that the anterior end was pointed, or else the pieces were kept flat in vaseline under water. Even so, scarcely any anterior regeneration took place. When the posterior end of the same piece was also cut off, it was rapidly regenerated, proving, as in *Drendrocœlum*, that growth in the anterior direction was not wanting because of a general lack of power to regenerate in the absence of the cephalic ganglia. The pieces were not kept long enough to prove that anterior regeneration never could occur, but, whatever the limit, the rate is excessively slow as compared with the rate in other directions or in other sorts of pieces. One worm which was supposedly cut behind the brain, completely regenerated the anterior end including ganglia and eye-spots. It is undoubtedly true that it was cut posterior to the eye-spots, but the probability is that part at least of the ganglia remained. That this would be possible can readily be seen from the figure of a horizontal section of a normal worm, showing the relative positions of ganglia and eye-spots.*

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[1] Lillie, F. R., '01, "Notes on Regeneration and Regulation in Planarians," *Am. Journ. of Physiology*, Vol. XI., p. 129.

[2] Morgan, T. H.,'04, "Notes on Regeneration," BIOL. BULLETIN, Vol. VI., p. 159.

[3] Schultz, E., '02, "Aus dem Gebiete der Regeneration, II., Ueber die Regeneration bei Turbellarien," *Zeitsch. der wiss. Zool.*, 72 Bd., p. 1.

[4] Child, C. M.,'04, "Studies on Regulation, IV., Some Experimental Modifications of Form-regulation in Leptoplana," *Journ. of Exp. Zool.*, Vol. I., p. 95.

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* As this paper goes to press, a new paper has appeared by C. M. Child, "Studies on Regulation, VI., The Relation Between the Central Nervous System and Regula-

So far then the results showed nothing different from what had already been observed in other forms, but the power to regenerate ganglia had been tested only in pieces from which the tissue anterior to the level of the ganglia had been cut away, and the question remained whether that power was entirely wanting under all conditions. The ganglia were accordingly removed in a number of ways, all differing from a cut straight across the worm in that some of the tissue anterior to the level of the ganglia in each case remained in the piece that was to regenerate. The worms were cut as follows :

1. The ganglia were removed with as much of the tissue anterior to them as was necessary to make the cut, leaving however parts of the anterior end of the worm on both sides of the cut (Fig. A, 1).

2. The ganglia were removed from the side, with as little as possible of the lateral tissue and leaving all of the anterior tissue (Fig. A, 2).

3. The ganglia were removed in a right-angled piece, leaving the lateral part of the anterior end on one side (Fig. A, 3).

4. The ganglia were cut out with a straw, leaving a round hole in the worm (Fig. A, 4).

5a. The worm was cut in half longitudinally, and the ganglia removed from the pieces, or the ganglia were first removed with a straw, and the worm then cut (Fig. A, 5a). 5b. A slight variation of 5a was made by removing the ganglia with a straw, and a few days later, when the wound had closed, cutting the worm longitudinally (Fig. A, 5b). 5c. The anterior half of one side was cut off, and the ganglion of the other side cut out (Fig. A, 5c).

In all of these cases, unless as in a few instances the wound healed in such a way as to prevent growth of new tissue, regeneration took place, and externally, the worm appeared to be rapidly attaining the normal condition. The worms were not always kept until the form was completly restored, but regenera-

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tion in Leptoplana : Anterior and Lateral Regeneration," *Jour. of Exp. Zool.*, Vol. I., p. 513, 1904. Among other results, he has found that anterior regeneration is complete when not much more than half of the cephalic ganglion is removed by a cross-cut.

tion was in every case rapidly taking place, and where checks were possible, pieces with and without ganglia were in the same condition. For example, in 5a, half-worms retaining one ganglion, kept in the same dish with those without ganglia, regenerated at about the same rate.

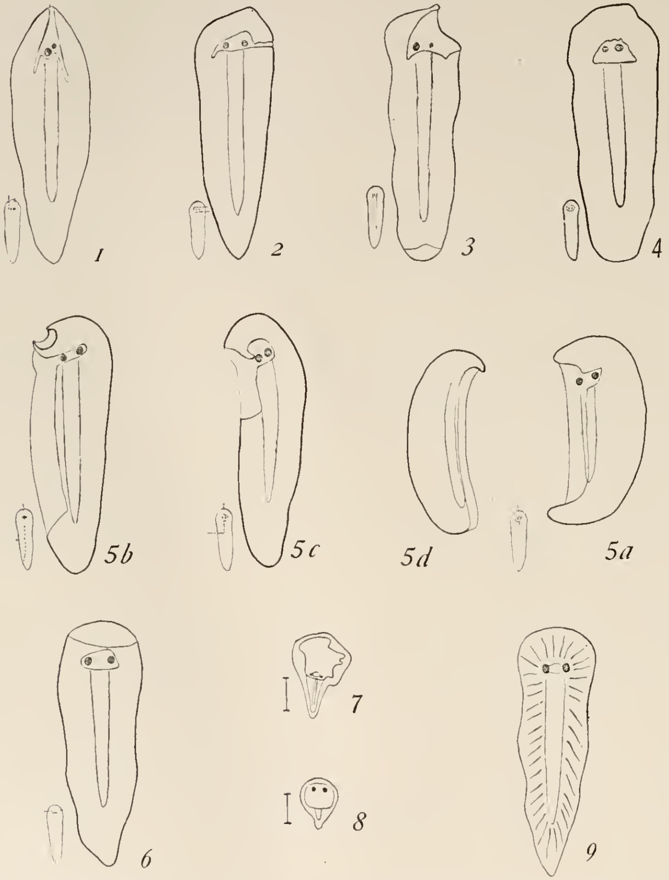

FIG. A, Diagrammatic figures of (1–6), worms regenerated after injury, indicated in each case by the accompanying small diagram. (7, 8), Regenerated pieces cut out of the head of the worm, and including cephalic ganglia and eye-spots. (9), Normal worm.

In the pieces deprived of ganglia, the cut surfaces fused together, new tissue was formed, and eye-spots appeared in about two or three weeks, often in two symmetrical groups, but sometimes with irregularities in size and position. The pieces at first were sluggish, and behaved like pieces from which the whole anterior end including the ganglia had been cut off, but as the eye-spots and other missing parts appeared the worms behaved more like the normal animal. In the light of what follows, it would be interesting to make more detailed observations on this point, for, in spite of the external signs of complete regeneration, sections show that the ganglia themselves have not regenerated. In the normal worm, the cephalic ganglia are well defined, and enclosed in a distinct sheath, and have the appearance in section of a brain of two hemispheres. The large nerve cords pass through the sheath and are connected with the ganglion (Fig. B, 1). In the regenerated worms, in place of the definitely defined cephalic ganglia, nerve fibers alone are present,

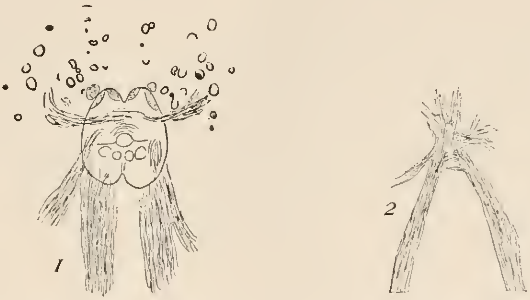

FIG. B (1), Diagrammatic figure of the cephalic ganglia, nerves and eye-spots of a normal worm, taken from a horizontal section. (2), Diagrammatic figure of the nerves in the position of the ganglia, in the regenerated worm of Fig. A (2), seven weeks after the removal of the cephalic ganglia.

which connect the large anterior and posterior nerve cords of the two sides of the body (Fig. B, 2). The eye-spots of the normal worm are more numerous and cover a larger area than in the regenerated worm. In the latter, the eye-spots are usually found in two well defined groups, though sometimes, crowded out of place or irregular, when the wound has healed in such a way as to leave too small a space for them to develop in the normal position.

In the absence of the ganglia it seems to be a fact that the presence of some anterior tissue is conducive to regeneration. It will be noted that in all the cases described where regeneration occurred without the ganglia, some of the old tissue anterior to the level of the ganglia was left in the piece. The difference in regeneration with and without some anterior tissue was strikingly shown in the following experiment : the cephalic ganglia were removed with a straw, and the worm then cut in half longitudinally, the left half through an accident was injured, and some of the anterior tissue broken off. After seven weeks, the right half had regenerated well, and had for two weeks shown two distinct eye-spots, as in numbers of other similar cases. The left side, kept in the same dish for the same length of time, showed less regeneration, and no eye-spots had appeared, nor could any be found in sections (Fig. A, 5*a*, 5*d*). In all the cases where regeneration occurred in the absence of the ganglia, the cuts were made in such a way that lateral regeneration without anterior regeneration might account for the restoration of the form of the worm.

Regeneration of the anterior tip of the worm, that is when the worm has been cut off anterior to the ganglia, occurs in the absence of the ganglia as well as when they are present. The ganglia of several worms were removed with a straw, and after a few days, when the wound had closed, the anterior end was cut off anterior to the scar. Regeneration occurred as in check experiments, where the ganglia were retained, and by the time the eye-spots had reappeared, the form of the worm was completed (Fig. A, 6).

SUMMARY AND CONCLUSIONS.

The experiments show that :

1. Pieces containing the cephalic ganglia behave like normal animals, and regenerate readily.

2. Pieces deprived of the ganglia are sluggish, and may or may not partially regenerate.

3. Pieces cut across at any level posterior to the ganglia, do not regenerate to any extent anteriorly ; but

4. Pieces deprived of the ganglia in such a way that some tissue anterior to the level of the ganglia remains in the piece,

regenerate partially. The normal form and behavior of the worm are restored, and the eye-spots are formed; but

5. The cephalic ganglia themselves never regenerate if completely removed.

6. Regeneration of the anterior tip of the worm occurs in the absence of the ganglia.

7. Lateral regeneration occurs in the absence of the ganglia, even though the ganglia themselves are not restored. It still remains unexplained why in the polyclads the tissue posterior to the cephalic ganglia does not regenerate the missing parts anterior to their level, and why the tissue around the ganglia does not restore them. It seems clear that the absence of the ganglia is in some way directly responsible for the lack of anterior regeneration, for when the ganglia are not entirely removed, complete regeneration occurs. The completion of the form of the worm and regeneration of eye-spots (though without the ganglia) in experiments 1–5 may have come about mostly through lateral regeneration, for in each case anterior tissue remained on one or both sides.

My work was carried out at the Marine Laboratory of Leland Stanford University. It gives me pleasure to express my appreciation of the courtesy extended to me while at Pacific Grove.

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BIOLOGICAL BULLETIN

THE ANATOMY OF THE EYES AND NEURAL GLANDS IN THE AGGREGATED FORMS OF CYCLOSALPA DOLICHOSOMA-VIRGULA AND SALPA PUNCTATA.[1]

MAYNARD M. METCALF AND MARY E. G. LENTZ JOHNSON, M.A.

(WITH PLATES VII., VIII. AND IX.)

The eyes of the solitary salpas of the several species show but little diversity. All are in the form of a horseshoe above the ganglion, the ends of the horseshoe pointing forward. In *Iasis cordiformis-zonaria* the ends of the horseshoe diverge, pointing outward and upward at an angle of about 45°. In *Salpa runcinata-fusiformis* there are masses of somewhat irregular rod-cells in the dorsal part of the ganglion, in front of the eye. With these exceptions the differences in the eyes of the solitary forms of the different species are too minute for verbal description.

In the aggregated forms, on the other hand, the diversity between the eyes is very great. Each species has its own characteristic and distinctive form of eye and the histological differences are great. In addition to the large dorsal eye some species have smaller eyes in the ganglion, and the number, position and character of these smaller eyes vary with the species. It is, therefore, important that we have recorded descriptions of the anatomy of the eyes in the chain individuals of all species. Especially is this true since suggestions as to relationship, so readily received from the study of the eyes, appear to be trustworthy.

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[1] This study of the eyes and neural glands of two species of *Salpidæ* was accepted in June, 1903, by the Woman's College of Baltimore, in partial discharge of the requirements for the degree Master of Arts at that time conferred upon Miss Mary E. G. Lentz. Its publication has been delayed until I could receive and study additional material of these and other species of Salpa in the hope of getting conclusive evidence upon disputed points of innervation. I obtained from Naples material of three species of Salpa preserved some in half strength glycerine and some in chloral hydrate, and endeavored to study the innervation of the eye by the use of dissociation media, especially Haller's acetic acid mixture, but the results obtained are not sufficiently conclusive to warrant publication.

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The neural glands of the chain forms of different species may also be very diverse, making it desirable to have descriptions of their anatomy in all species.

In the present paper the anatomy of the eyes and of the neural glands will be described for the chain forms of two species whose eyes have not before been studied—*Clycosalpa dolichosoma-virgula* and *Salpa punctata*.

The other salpas the anatomy of whose eyes has been studied are :

Cyclosalpa pinnata, solitary and chain forms (by Göppert and Metcalf).

C. chamissonis, chain form (by Metcalf).

Salpa africana-maxima, solitary and chain forms (by Göppert and Metcalf).

S. runcinata-fusiformis, solitary and chain forms (by Göppert and Metcalf).

S. cylindrica, solitary and chain forms (by Metcalf).

Iasis hexagona, solitary and chain forms (by Metcalf).

I. costata-tilesii, solitary and chain forms (by Metcalf).

I. cordiformis-zonaria, solitary and chain forms (by Metcalf).

Pegea scutigera-confederata, solitary and chain forms (by Göppert and Metcalf).

P. scutigera-confederata var. *bicaudata*, chain form (by Metcalf).

Thalia democratica-mucronata, solitary and chain forms (by Göppert and Metcalf).

The anatomy of the neural glands has been described by Metcalf for the chain and solitary forms of the species mentioned in the foregoing list, except that the gland has not been described for the solitary *Pegea scutigera-confederata* var. *bicaudata*. It is probable that *bicaudata* is a variety of the chain form only and that its solitary form resembles that of *scutigera-confederata*.

The development of the eyes and neural glands has been described by Metcalf for *Cyclosalpa pinnata*, chain and solitary forms.

CYCLOSALPA DOLICHOSOMA-VIRGULA, chain form.

The condition of the eye in this species is of interest because it forms the third link in a series of three conditions bridging over the gap between the *Cyclosalpæ* and the true *Salpæ*. The structure of its neural glands is not unique.

The brain of *Cyclosalpa dolichosoma-virgula* is a ganglion of almost spherical shape (Fig. 1). Two outgrowths from the ventral surface, one on either side of the mid-line, extend to the neural glands whose position is ventro-lateral to the ganglion. These glands, with their long lateral ducts, are partially shown in Fig. 1 (*h*, gland; *d*, duct). On the postero-dorsal surface of the ganglion is a slight protuberance (Fig. 5, *pr*). All the nerves, with the exception of a single pair, spring from the equatorial zone of the ganglion (Fig. 1). One pair arises from the ventral outgrowths. The nerves are probably constant in number for the species — forty-eight.

The large ovoid eye (Fig. 1) projects anteriorly from the posterior end of the dorsal surface of the ganglion, and the long axis of the eye makes with this surface an angle of about 30° (Fig. 5). The eye points about 40° from the mid-line of the body, to the right or left, according as the animal lies to the right or left in the chain. One third of the eye, approximately, extends beyond the anteriormost edge of the ganglion.

There is a deep invagination of the ectoderm into the narrow space between the eye and the ganglion, and a less abrupt invagination just posterior to the ganglion (Fig. 5). In the ectodermal chamber formed between these two invaginations lies the eye, which presses closely against the ectoderm only at its tip. Blood sinuses (Fig. 1, *bl*) which surround the ganglion are continuous with this optic chamber (Fig. 5, *oc*).

The ganglion has the usual fibrous core, and a peripheral cellular layer (Fig. 5), which extends inward about one seventh of the diameter. The postero-dorsal protuberance (Fig. 5, *pr*), is made up of ordinary ganglionic cells. Large nerve cells (Fig. 5, *nc*), lie in the equatorial zone from which the nerves arise.

These large cells lie among the smaller ganglionic cells of the peripheral layer. A large bundle of nerve fibers, arising in the fibrous core of the ganglion, passes through the peripheral cellular layer, just anterior to the protuberance on the posterior surface, and enters the eye at its posterior end, forming the optic nerve (Fig. 5, *on*). Fibers ramify also among the cells of the postero-dorsal protuberance (Fig. 5, *pr*).

Four kinds of cells are found in the large eye, and one of these, so far as known, is peculiar to this species. The pigment cells and rod-cells resemble the characteristic cells found in the well-developed eyes throughout the genus. The pigment cells cover the dorsal surface of the eye from near the tip to slightly beyond the middle (Fig. 1 and Fig. 5, *dp*). They then extend around the lateral surfaces in posteriorly-directed broad bands, uniting on the ventral surface to form a pigment layer which extends from beyond the middle of the eye to the base (Fig. 5, *vp*). The position of the dorsal and ventral pigment is also shown in cross sections (Fig. 7, *dp*; Fig. 9, *vp*). A cross section through the middle of the eye (Fig. 8), shows the complete enwrapping of this portion of the eye by pigment. The pigment cells lie outside the optic membrane (Fig. 5, *oz*), which is a continuation of the ganglionic membrane. They are therefore probably mesodermal.

The rod-cells are elongated cells with large nuclei (Fig. 5, *dr* and *vr*). They have thick-walled basal ends and thin-walled tips. In the anterior and posterior regions of the eye the thick-walled ends of the rod-cells are toward the pigment (Figs. 5, 7 and 9). In the center of the eye, where the pigment forms a continuous enveloping layer, the thick-walled inner ends of the rod-cells have a somewhat confused arrangement, while their thin-walled tips point toward the pigment cells (Figs. 5 and 8).

The third kind of cells present in the eye are similar to the ordinary cells of the ganglion, having nuclei of about the same size. These cells, which have been called intermediate cells, lie between the thick-walled ends of the anterior and posterior rod-cells and the pigment cells (Fig. 5, *i* and *i′*; Fig. 7, *i*; Fig. 9, *i′*). Intermediate cells are not present in all species. In the chain forms of *Cyclosalpa pinnata* and *Pegea scutigera-confederata* the rod-cells abut directly on the pigment layers.

The large optic nerve, when it enters the eye, passes directly above the thin-walled ends of the rod-cells in the posterior portion of the eye (Fig. 5; Fig. 9, *on*). The nerve can easily be traced as far as the middle of the eye, but the course of the fibers beyond that point could not be satisfactorily determined. In sections through the tip of the eye, the nerve fibers seem to appear ventral to the rod-cells (Fig. 5), and it seems probable that they enter these cells at their thin-walled ends; but the shrinking of the protoplasm from the tips of the rod-cells, apparently due to the action of the preserving fluids, makes the rod-cell walls more clearly visible, so that it is very difficult to distinguish between these cell boundaries and probable innervating fibers. Sufficient evidence was not obtained to warrant a categoric denial of Göppert's assertion that the rod-cells in the anterior portion of the eye, in the chain forms, receive their fibers at their thick-walled ends, though the indications are all against this belief. To conclusively decide this disputed point of innervation, attempts were made at macerating preserved material, but without success. Any one able to obtain fresh specimens could probably determine readily by maceration the manner of innervation. Best suited for this purpose are the following species, in which the rod-cells are well-developed: *Cyclosalpa pinnata*, *Cyclosalpa chamissonis*, *Cyclosalpa dolichosma-virgula*, *Salpa runcinata-fusiformis*, *Salpa africana-maxima*, *Salpa cylindrica*, *Thalia democratica-mucronata*, *Salpa punctata*.

The fourth kind of cells, found in the eye of the chain form of *Cyclosalpa dolichosoma-virgula*, are those which have been mentioned as perhaps peculiar to this species. They lie in a single group, which is partially imbedded among the pigment cells of the mid-dorsal region (Fig. 5, *q*; Fig. 8, *q*; enlarged, Fig. 10, *q*). These cells are spindle-shaped, and have nuclei about the size of the intermediate-cell nuclei. They are inclined at an angle of about 45° to the long axis of the eye (Fig. 10), and they are separated from the posterior rod-cells by the optic nerve fibers. The anteriormost cells of the group are wholly surrounded by pigment (Fig. 8, *q*). There seems to be some indication that these spindle-shaped cells receive at their posterior ends innervating fibers from the optic nerve. A probable homology of this group of cells with certain portions of the eye of *Cyclosalpa pinnata* and *Cyclosalpa chamissonis* will later be discussed.

Small groups of apparent rod-cells are found in the peripheral cellular layer of the ganglion, (Fig. 5, *cy*, *cy′*, *cy″*). They may perhaps be called small eyes, because they are made up of cells with thick-walled basal ends, similar to the rod-cells of the large eye. An enlarged drawing of one of these groups is shown in Fig. 11. The cells of these small eyes are not elongated as are the rod-cells of the large eye, and their nuclei are the same size as those of the ordinary ganglionic cells. More or less similar smaller eyes occur in the ganglia of the chain forms of *Cyclosalpa pinnata*, *C. chamissonis*, *Salpa cylindrica*, *S. runcinata-fusiformis*, *Iasis hexagona*, *I. costa-tilesii*, *Pegea scutigera-confederata* and *Thalia democratica-micronata*. Compare Fig. 18, Plate IX., which shows their position and appearance in *Cyclosalpa pinnata*.

There is no pigment present in these small eyes of any species described except *Salpa costata-tilesii*. Göppert assumes that these eyes are functional optic organs, but the absence of pigment makes the correctness of this assumption doubtful.

The small groups of rod cells which occur in the ganglion of *Cyclosalpa dolichosoma-virgula* vary in number and position, but all have about the same structure. Their cells are about the same size as the ordinary peripheral cells of the ganglion and seem to be developed from these merely by the formation of the peculiar outer glassy layer indicated in the figures (Plate VIII., Figs. 5 and 11) by the heavy black lines. The glassy outer portion of one of these cells resembles in histological character the glassy outer layer, which we have called the thickened cell-wall, seen at one end of any rod-cell of the larger eye, but the cells are of quite different shape, being spheroidal, or irregularly polyhedral, instead of cylindrical. There seems to be a general tendency in the chain forms of the different species of *Salipidæ* to form from the smaller cells of the ganglion such groups of imperfect rod-cells.

Each of the two latero-ventral outgrowths which push out from the ganglion toward the glands consists of cells of two sizes.

The ordinary ganglionic cells form that portion next to the ganglion (Fig. 6, *b*). A distinct though thin membrane then intervenes (Fig. 6, *gz*), separating the small-celled portion from a large-celled area (Fig. 6, *b′*), which extends to the wall of the gland (Fig. 6, *gw*), and is separated from the gland by a thick membrane (Fig. 6, *hz*). The thin membrane, which intervenes between the small and large cells of the outgrowths is continuous with the delicate membrane that surrounds the ganglion. The neural glands and their lateral ducts have walls made up of a single layer of cells, except that the wall of that side of each gland which lies next to the ganglionic outgrowth, is composed of several layers of cells (Fig. 6, *gw*). These conditions are very similar to those described for *Cyclosalpa pinnata.*

SALPA PUNCTATA.

The ganglion of the chain form of *Salpa punctata* (Fig. 12) differs in shape from the nearly spherical ganglion of *Cyclosalpa dolichosoma-virgula.* Its dorso-ventral axis is about one and one half times the length of its anterior-posterior and transverse axes. Twenty-seven nerves were counted (Fig. 2). As in *Cyclosalpa dolichosoma-virgula,* they all arise from the equatorial zone of the ganglion, with the exception of a single pair from the ventral outgrowths. The average thickness of the peripheral cellular layer (Fig. 12), is about one twelfth of the ganglion's mean diameter.

In a dorsal position, just beneath the ganglionic membrane, is a double layer of rod-cells, with the thickened ends of the cells of one layer abutting upon the thickened ends of those of the other layer (Fig. 12, *cx*). These cells are not as elongated as the rod-cells of the large eye, but their bulk is about the same and their nuclei are about the same size. There are no scattered groups of small rod-cells in the peripheral cellular layer of the ganglion of *Salpa punctata,* such as have been described for *Cyclosalpa dolichosoma-virgula.* This group of rod-cells in the dorsal part of the ganglion of *Salpa punctata* probably belongs to the same category as the small eyes found in the ganglia of many species. The presence of many sorts of these small groups of rod-cells in different positions in different species renders homologies between them doubtful.

The large eye projects from the antero-dorsal face of the ganglion, and therefore lies almost wholly anterior to the ganglion. Its projection to the right or left of the mid-line of the body is slightly less than that of the eye of *Cyclosalpa dolichosoma-virgula.* The eye is relatively short, and tapers but slightly toward the tip. The dorsal pigment layer extends more than half way down over the tip (Figs. 12 and 13, *dp*), thus enveloping the dorsal half of the anterior portion of the eye in a hood of pigment. The lateral posteriorly-directed pigment bands (Fig. 15, *lp*), meet at the postero-ventral angle of the eye to form the ventral pigment surface, which is of less extent than the dorsal (Fig. 12, *vp*). Slightly posterior to the middle of the eye the dorsal pigment dips down forming a pigment curtain (Fig. 12, *pc*), which extends nearly half way through the eye, and is laterally continuous with the lateral pigment layers (Fig. 14). This pigment curtain is slightly dome-shaped with the apex of the dome toward the anterior. Fig. 14 shows a section through the pigment curtain just posterior to its apex.

The rod-cells have the same general arrangement as in *Cyclosalpa dolichosoma-virgula*, but in *Salpa punctata* those pointing ventrally (Fig. 12, *vr*) exceed in number those pointing dorsally (Fig. 12, *dr*). This corresponds to the fact already mentioned that the greater amount of pigment is on the dorsal surface. In the middle of the eye, where it is entirely enwrapped by pigment, the rod-cells point both dorsally and ventrally, with their thick-walled ends central. None point laterally, as in *Cyclosalpa dolichosoma-virgula* (compare Fig. 8, *lr*). The optic nerve enters the eye above the thin-walled tips of the posterior rod-cells (Fig. 12, *on*), and the innervation is believed to be the same as that of *Cyclosalpa dolichosoma-virgula.*

A large group of intermediate cells lies between the dorsal pigment and the ventrally-directed rod-cells (Fig. 12, *i*). A smaller group lies between the ventral pigment and the few dorsally-directed rod-cells (Fig. 12, *i'*). In *Cyclosalpa dolichosoma-virgula* the intermediate cells are confined to those two regions, but in the *Salpa punctata* the intermediate cells extend also beneath the lateral pigment layers. The position of the lateral intermediate cells in *Salpa punctata* is the same as that of the laterally-directed rod-cells of *Cyclosalpa dolichosoma-virgula* (compare Fig. 8, *lr*), and these cells in the two species are probably homologous, the rod-cells in *Cyclosalpa dolichosoma-virgula* having developed from intermediate cells. A few intermediate cells lie posterior to the dorsal pigment curtain (Fig. 12, *q'*). The group of spindle-shaped cells in the eye of *Cyclosalpa dolichosoma-virgula* occupies a similar position (compare Fig. 5, *q*).

The ectoderm (Fig. 12, *os*), closely covers the entire eye and dorsal surface of the ganglion, except at the postero-dorsal angle of the ganglion. A membrane (Fig. 12, *z*), continuous with the ganglionic membrane, meets the ectoderm posterior to the ganglion, shutting off a small chamber (Fig. 12, *oc*), which is homologous with the posterior portion of the optic chamber of other species, such as *Cyclosalpa dolichosoma-virgula.* The limiting membrane of the optic chamber (Fig. 12, *z*), described for *Salpa punctata* is not found in *Cyclosalpa dolichosoma-virgula.* It is present in many other species, and its absence in *Cyclosalpa dolichosoma-virgula* is exceptional.

The ectoderm, after covering the eye and ganglion, turns abruptly dorsalward, so that the eye and ganglion lie just under an ectodermal invagination. This invagination thus forms a supra-neural ectodermal chamber which is filled by an unusually dense portion of the tunic (Fig. 12, *sc*). It has a somewhat triangular dorsal opening to the exterior. The supra-neural ectodermal chamber in *Salpa punctata* probably serves for the eye and ganglion a similar protective purpose to that of the large optic chamber of *Cyclosalpa dolichosoma-virgula.* A similar ectodermal invagination is found above the ganglion of the immature *Salpa runcinata-fusiformis*, chain form.

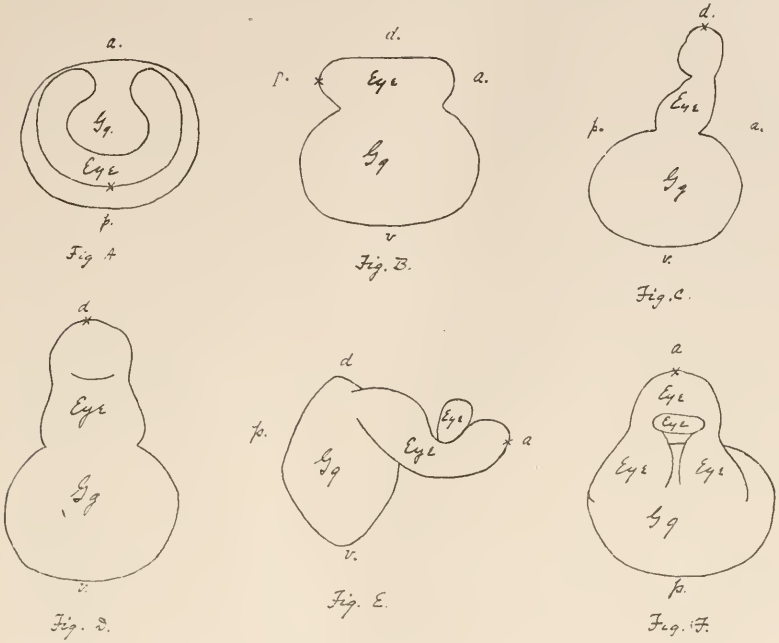
The latero-ventral outgrowths from the ganglion to the neural glands are similar in structure to those of *Cyclosalpa dolichosoma-virgula*, but smaller. The wall and duct of each gland are composed of a single layer of cells. That portion of the wall next to the ganglion is thickened by the elongation of the cells but these cells even here form a single layer. This is unlike the condition found in *Cyclosalpa dolichosoma-virgula*, in which the thickening of the gland wall next to the ganglion is caused by the presence of several layers of cells. The gland ducts of

Salpa punctata are very broad and flattened, and show in cross-section a slit-like lumen. They are convoluted at their distal apertures (Fig. 16).

The eyes in the three cyclosalpas, *pinnata*, *chamissonis*, and *dolichosoma-virgula*, show an interesting series of modifications. Figs. 3 and 4, Plate VII., show the character of the large eye in *C. pinnata*. It is roughly a horseshoe with the ends directed backward, and having an additional mass of optic cells placed in the curve of the horseshoe. This eye has been shown (by Metcalf) to be derived from a horseshoe-shaped eye, like that of the solitary salpa, whose free ends point forward. The position of the eye has been reversed by an actual tipping forward of the whole eye, the ends of the horseshoe remaining attached to the ganglion, while the curved part of the horseshoe swings forward through an arc of 180° (*cf.* Text-figs. 1 to 6). The horseshoe shape of the adult eye of the chain *C. pinnata* is therefore probably in a sense primitive, a reminder of the condition seen in the embryo, which corresponds to that in all the solitary salpas. For a time, however, in the development of the chain *C. pinnata* the young eye is undivided, there being no split between the lateral halves. This split, which appears later, is probably a reopening of the earlier space between the limbs of the horseshoe seen at a time when in the young embryo the eye had a form similar to that of the eye of the adult solitary salpa.

Metcalf has shown that the eye of the chain *Cyclosalpa chamissonis* is in a condition a little less developed than that of *C. pinnata*. It is in the form of a flat plate, with no split between its two halves (Pl. IX., Fig. 19), though there are two enlargements of its posterior portion, corresponding to the two limbs of the eye of *C. pinnata* and separated by a furrow which is in the position occupied by the split in the eye of *C. pinnata*. *C. chamissonis* has also an accessory mass of optic cells dorsal to the anterior part of the eye in the same position in which the similar body is seen in *C. pinnata* (compare Fig. 19, r''' with Fig. 18, p''' and c'''). The eye of *C. chamissonis* is either less developed or has reverted to a slightly simpler condition.

The eye of *Cyclosalpa dolichosoma-virgula* (Pl. VII., Fig. 1, and Pl. VIII., Fig. 5) is still more different from that of *C. pinnata*. It is irregularly conical rather than flat and shows no sign of division into right and left limbs, approaching in form and structure the eye of the true *Salpæ*, for example *S. runcinata-fusiformis.*



Outline drawings of ganglia and eyes of salpa.

a. = anterior; *d.* = dorsal; *Gg.* = ganglion; *p.* = posterior; *v.* = ventral; *x.* indicates in each case the same region of the eye.

FIG. A. Dorsal view of ganglion and eye of a solitary salpa. The horseshoe-shaped eye is above the ganglion, with the ends of the horseshoe pointing forward.

FIG. B. The same seen from the right side.

FIG. C. Ganglion and eye of an immature chain *Cyclosalpa pinnata*. In an earlier condition this eye was horseshoe-shaped and lay horizontally on the dorsal surface of the ganglion, resemblimg Figs. A and B. It has now rotated forward and upward through an arc of about 90°. That surface of the eye which was dorsal when the eye was horizontal is now anterior.

FIG. D. The same eye and ganglion as those shown in Fig. C., but here seen from behind. The eye is in the form of a flat plate, the hollow of the horseshoe having become filled with cells.

FIG. E. The eye and ganglion of an adult, chain *Cyclosalpa pinnata*, seen from the right side. The eye has shifted 90° still further forward and is again horizontal, but with its former posterior portion now anterior. (Cf. Plate VII., Fig. 3.)

FIG. F. Dorsal view of the same eye and ganglion as those shown in Fig. E. The eye is seen to be spiit posteriorly into two limbs, being again horseshoe-shaped. (Cf. Plate VII., Fig. 4.)

The protuberance from the ganglion, which lies at the base of the large eye, is very similar to that seen in *C. pinnata* (*cf.* Figs. 5 and 18). In the eye of *C. dolichosoma-virgula* there is a group of cells which strongly suggests comparison with the accessory mass of optic cells in the curve of the horseshoe in the eye of the chain *C. pinnata* and with the similar, though less distinct mass of cells in *C. chamissonis*. This mass of peculiar spindle-shaped cells does not show in surface view, but is shown as it appears in section in Pl. VIII., Figs. 5 and 10, *q*. They are not developed as rod-cells, yet their shape, their position, their innervation, and their relation to the pigment cells seem to indicate that they are to be compared to the accessory portion of the eyes of the other *Cyclosalpæ*. Yet, in spite of the presence of this peculiar group of cells, the eye of *C. dolichosoma-virgula* resembles those of the true *Salpæ* more than it does the eyes of the other *Cyclosalpæ*. If the *Cyclosalpæ* are more primitive than the *Salpæ*, as many structural features seem to indicate, the study of the eye of *C. dolichosoma-virgula* would suggest a transitional stage from one group to the other.

Another resemblance between *Cyclosalpa dolichosoma-virgula* and the true *Salpæ* is seen in the fact that in the former species and in *Salpa runcinata-fusiformis* there is found above the ganglion a peculiar ectodermal invagination which opens dorsally by a relatively narrow mouth, and which probably serves as a protection for the ganglion and eye, no large optic chamber being present in either of these species.

The position of the large eye in the chain salpas is worth noting. In most species the eye projects upwards (*Salpa runcinata-fusiformis*, Fig. 20, Pl. IX.) or forward from the dorsal face of the ganglion (compare Fig. 5, Pl. VIII., which shows a median section of the ganglion and eye of *Cyclosalpa dolichosoma-virgula*). In *Salpa punctata* the eye projects slightly downward from the antero-dorsal surface of the ganglion (Fig. 12, Pl. IX.). In *Thalia democratica-mucronata* the eye projects downward and backward from the antero-ventral surface of the ganglion (Fig. 17, Pl. IX.). These conditions, and the manner in which in *Thalia* the ectoderm is drawn down in front of the ganglion, indicate that the whole ganglion with the eye has rotated forward and downward. Compare Figs. 5, 12 and 17, and note that in

Cyclosalpa dolichosoma-virgula the positions of these parts is the usual one; in *Salpa punctata* the shifting has been slight, about 45 °, in *Thalia* the rotation is greatest, 180 ° or more. The position and arrangement of the nerves in *Thalia* confirms this interpretation.

Remembering the rotation that has occurred in *Salpa punctata*, it seems clear, on comparing Fig. 5, Pl. VIII., and Fig. 12, Pl. IX., that the group of rod-cells lying behind the origin of the optic nerve, in the dorsal part of the ganglion, in *S. punctata* corresponds to the postero-dorsal protuberance from the ganglion in *Cyclosalpa dolichosoma-virgula*, although in the latter species rod-cells are not found in this region. There is great diversity between species in the extent to which groups of rod-cells are developed in the periphery of the ganglion. A similar postero-dorsal protuberance containing no rod-cells is found in *Cyclosalpa pinnata* (Fig. 18, Pl. IX.).

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EXPLANATION OF PLATES.

a, accessory mass of optic cells ; *b*, small cells of latero-ventral outgrowths from ganglion ; *b'*, large cells of latero-ventral outgrowths from ganglion ; *bl*, blood sinus ; *d*, duct of neural gland ; *dp*, dorsal pigment cells of large eye ; *dr*, dorsally-directed rod-cells of large eye ; *e'''*, "accessory portion" of *Cyclosalpa pinnata* eye ; *ec*, outline of ectodermal chamber above the ganglion ; *ex*, dorsal group of rod-cells in ganglion of *Salpa runcinata-fusiformis* and *Salpa punctata ;* in Fig. 17 it indicates a group of optic cells in *Thalia democratica-mucronata* which may be homologous with the cells marked *ex* in the *Salpæ* (cf. Figs. 12 and 20, Plate III.); *ey*, *ey'*, *ey''*, groups of rod-cells in peripheral cellular layer of ganglion of *Cyclosalpa dolichosoma-virgula ; ez*, a group of optic cells in *Thalia democratica-mucronata* which is apparently homologous with one of the posterior limbs of the horseshoe-shaped eye of the chain form of *Cyclosalpa pinnata ; g*, ganglion ; *gc*, fibrous core of ganglion ; *gz*, membrane surrounding ganglion ; *h*, neural gland ; *hz*, membrane separating gland wall from large cells of latero-ventral outgrowths ; *i*, dorsal intermediate cells of large eye ; *i'*, ventral intermediate cells of large eye ; *l*, peripheral cellular layer of ganglion ; *lp*, lateral pigment cells of large eye ; *lr*, laterally-directed rod-cells of large eye ; *n*, nerve ; *nc*, nerve cells ; *o*, opening of duct of neural gland into the pharynx ; *oc*, optic chamber ; *on*, optic nerve ; *os*, optic ectodermal sheath ; *oz*, optic membrane covering eye ; *p'''*, pigment cells of "accessory portion" of *Cyclosalpa pinnata* eye ; *pc*, pigment curtain in *Salpa punctata* eye ; *pr*, postero-dorsal protuberance on ganglion of *Cyclosalpa dolichosoma-virgula* ; *q*, group of spindle-shaped cells in mid-dorsal region of *Cyclosalpa dolichosoma-virgula* eye ; *q'*, group of cells posterior to pigment curtain in *Salpa punctata* eye ; *r'''*, group of rod-cells in eye of *Cyclosalpa chamissonis* ; *sc*, supra-neural ectodermal chamber of *Salpa punctata* ; *vl*, ventro-lateral outgrowth ; *vp*, ventral pigment cells of large eye ; *vr*, ventrally-directed rod-cells of large eye ; *gw*, thickened portion of gland wall ; *x*, nerve which arises from one of the ventro-lateral outgrowths ; *y*, outline of optic chamber ; *z*, limiting membrane of optic chamber.

EXPLANATION OF PLATE VII.

FIG. 1. Dorsal view of eye, ganglion and nerves of *Cyclosalpa dolichosoma-virgula* chain form, showing also neural glands and ducts. The pigment of the eye is represented by heavy stippling. The ends of the rod-cells when visible are indicated by faintly stippled outlines. The outlines of some of the blood sinuses are indicated by dotted lines. The extent of the optic chamber is indicated by a contour-line.

FIG. 2. Dorsal view of the eye, ganglion, nerves, glands and ducts of *Salpa punctata*. The pigment of the eye is represented by heavy stippling. The rod-cells in the dorsal part of the ganglion are indicated by faintly stippled outlines. The rod-cells of the posterior portion of the large eye lie below so thick a layer of nerve fibers that their outlines do not show.

FIG. 3. The eye, ganglion, nerves and gland of the chain form of *Cyclosalpa pinnata* seen from the right side. The heavy stippling indicates pigment. The faintly stippled outlines indicate the contours of the rod-cells. The contour-line indicates the extent of the optic chamber. (Copied from Metcalf.)

FIG. 4. Dorsal view of the same. (Copied from Metcalf.)

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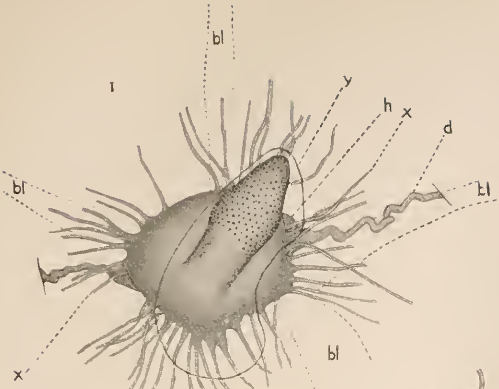
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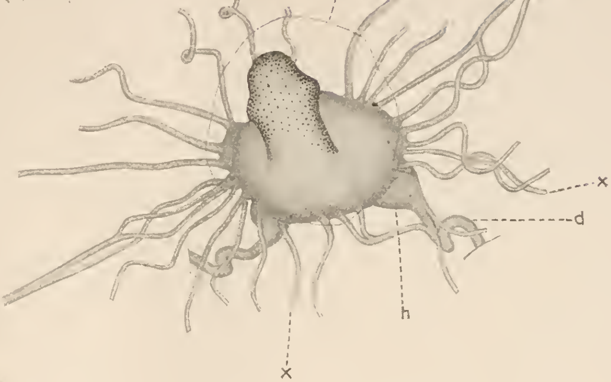

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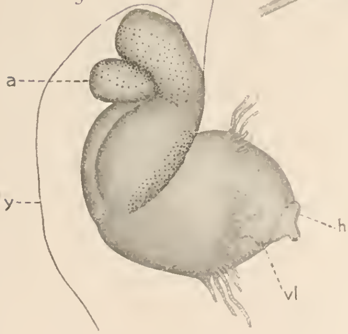

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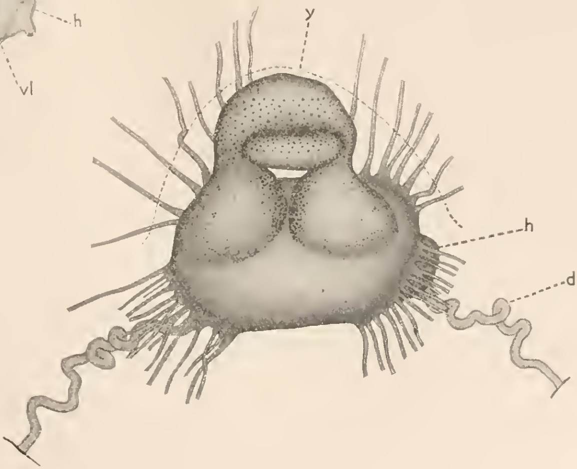

EXPLANATION OF PLATE VIII.

FIGS. 5–11. *Cyclosalpa dolichosoma-virgula*, chain form. Compare Fig. 1.

FIG. 5. Longitudinal vertical section of eye and ganglion, compounded from three oblique sections.

FIG. 6. Longitudinal section through the latero-ventral outgrowths from the ganglion and through one neural gland.

FIG. 7. Cross-section through the eye near the tip.

FIG. 8. Cross-section through middle of eye.

FIG. 9. Cross-section through eye near the base.

FIG. 10. Enlarged drawing of the section of the middle portion of the eye shown in FIG. 5, to show group of spindle-shaped cells, *q*.

FIG. 11. Enlarged drawing of one of the groups of rod-cells in the peripheral cellular layer of the ganglion.

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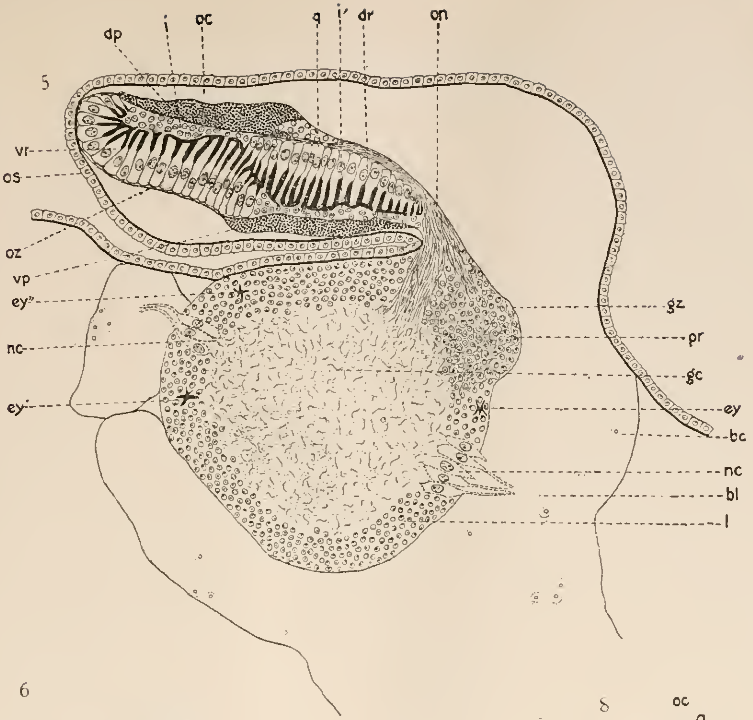
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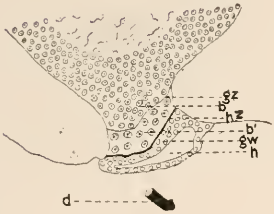
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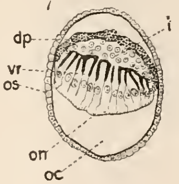
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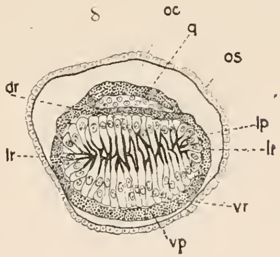
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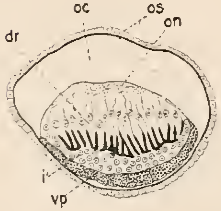
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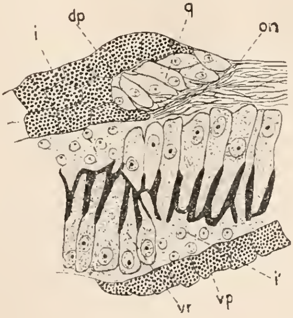
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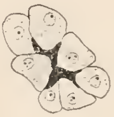

EXPLANATION OF PLATE IX.

FIGS. 12–16. *Salpa punctata*, chain form. Compare Fig. 2.

FIG. 12. Longitudinal vertical section of eye and ganglion.

FIG. 13. Cross-section through the eye near the tip.

FIG. 14. Cross-section through the middle of the eye.

FIG. 15. Cross-section through the eye near the base. In a section still further back the pigment would appear ventrally as well as laterally.

FIG. 16. Horizontal section through the distal end of the duct of one neural gland, showing convolutions at the aperture of the duct.

FIG. 17. Longitudinal vertical section of the eye and ganglion of *Thalia democratica-mucronata*, chain form (copied from Metcalf).

FIG. 18. Longitudinal vertical section of the eye and ganglion of *Cyclosalpa pinnata*, chain form (copied from Metcalf).

FIG. 19. Oblique (horizontal-cross) section through the middle of the eye of *Cyclosalpa chamissonis*, chain form (copied from Metcalf).

FIG. 20. Longitudinal vertical section of the eye and anterior part of the ganglion of *Salpa runcinata-fusiformis*, chain form (copied from Metcalf).

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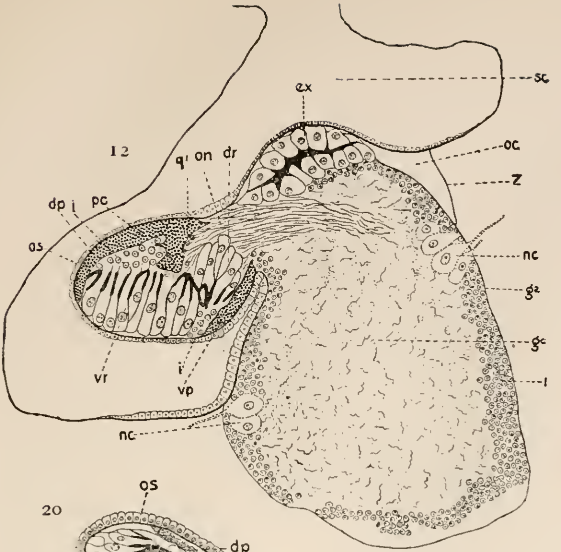
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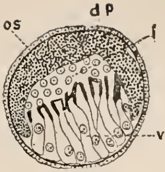

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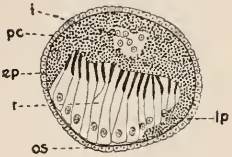
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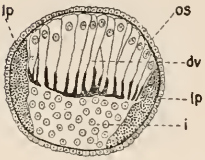

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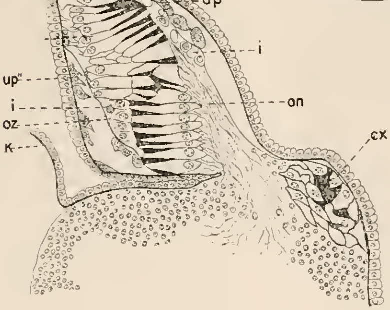
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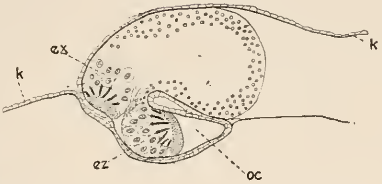

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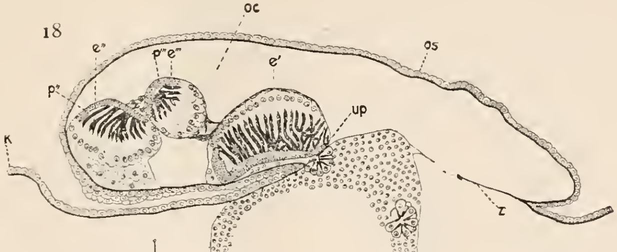
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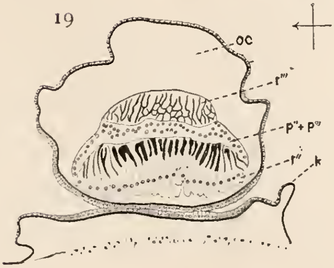

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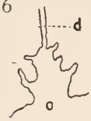

THE EXCRETORY AND CIRCULATORY SYSTEMS OF CRYPTOCHITON STELLERI MIDD.

HAROLD HEATH.

Somewhat over half a century ago Middendorff ('49) published his extensive studies on the anatomy of *Cryptochiton stelleri*, the largest and in some respects the most highly modified of the chitons. In several important details this was a decided contribution to our knowledge of the group and yet the work has not received the attention it has merited chiefly for the reason that the facts are inextricably associated with numerous errors (due to poorly preserved material) and are illustrated by figures often difficult to interpret. It is the purpose of the present paper to explain and to a certain extent correct Middendorff's results and especially to describe some of the more noteworthy features of the excretory and circulatory systems. This latter disvision of the subject may appear unnecessary owing to the extensive chiton studies already published, notably those of Haller ('82), Pelseneer ('99), Thiele ('02), and especially Plate ('97), but as will be noted in the following pages this species differs in several fundamental particulars from any other known form.

The specimens on which Middendorff's studies were based came from the shores of Kamchatka and from that locality this species extends to the southward fully 2,500 miles, the southern limit being approximately Monterey Bay, California. Throughout the greater part of their range, as I know from personal observation, they are confined to the littoral zone, rarely extending into water more than three fathoms in depth. Usually they are more or less concealed among the red algæ (especially *Gigartina exasperata*) upon which they feed and with which they harmonize so closely that they escape the untrained eye. While the largest northern specimens in my possession measure less than 20 cm. (8 inches) in length, some in the vicinity of San Francisco not infrequently are 25 cm. (10 inches) long. The largest specimen I have ever seen measured slightly more than

33 cm. (13 inches) when alive and in a resting condition and weighed seven grams less than two kilograms (4.4 lbs.).

Regarding the breeding habits little may be said at the present time. On three different occasions I have found males shedding their sex products in the latter part of February and at this time the females are distended with eggs. The oviduct is provided with an albumen gland in all essential respects like that of *Ischnochiton magdalenensis* (Heath, '99) and it is reasonable to suppose that like this last-named species *Cryptochiton* lays its eggs imbedded in a gelatinous envelope. In the early part of the summer the young have attained a length of from 10 to 22 mm., and as has been described in a previous paper (Heath, '97) the shells are still exposed. The mantle of these small individuals is usually of a yellow or orange color, exceptionally light green, and is beset with more or less definitely arranged yet scattered groups of crimson spicules characteristic of the adult. As these increase in number the general shade ordinarily changes to a brick red[1] not infrequently blotched with patches of white or purplish-white that usually disappear by the time the animal has become sexually mature.

Middendorff was the first to discover the kidney in the chitons yet it was with some hesitancy that he applied this name to it, as his observations were very incomplete. However, it must be said they are more perfect than some of his critics have supposed. He correctly states that the excretory canals unite in front of the pericardium in "einem geschlossenen Bogen" (p. 73) and he accurately locates the glandular portion, but the fact was never discovered that this latter division is penetrated by two canals, one of which connects with the pericardium while the other opens to the exterior.

The kidney holds the usual position at the sides of the visceral cavity and posseses the form of a very slender U, the free extremities terminating in the reno-pericardial, and external openings, while the opposite rounded end is situated at the level of the anterior margin of the third valve of the shell (Fig. 1).

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[1] Middendorff described his specimens as yellowish brown but this was due to the fact that the tufts of bristles had been worn away exposing the mantle to an abnormal degree.

At the extreme lateral border of the percardium and about one fourth its greatest length from its anterior end the relatively large inner opening occurs (Fig. 2, *r*) and almost immediately leads into a flat disc-like cavity closely attached to the ventral pericardial wall. From the inner border of this flattened sac a slender delicate tube, usually almost invisible in preserved material, proceeds forward and becomes continuous with the more dorsal tube of the glandular portion of the kidney proper. This last-named section consists, as in chitons generally, of numerous highly branched lobules which extend ventrally some distance on the inner surface of the foot, dorsally to the valves of the shell and to the head cavity in front.

In a number of species of chitons the outer limb of the excretory canal expands into a well defined reservoir (Nierensack), which is also supplied with glandular outgrowths. In some cases these reservoirs are of comparatively large size and in a few cases they may extend close to the mid-line, but here, as in every species of chiton hitherto described, one kidney is wholly independent of the other. In *Cryptochiton*, on the other hand, the reservoirs, that are probably distinct in very young specimens, unite in the mid-line and form, as Figs. 1 and 2 show a spacious chamber lying ventral to the anterior third of the pericardial cavity. In specimens one fourth the adult size, and occasionally in individuals of much greater length, there are slight indications of a median partition that may represent the line of fusion of the once independent divisions.

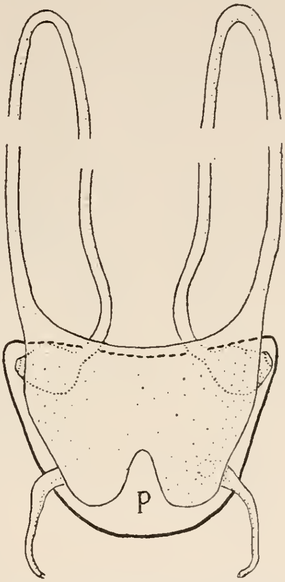


Fig. 1. Diagram illustrating the kidney of *C. stelleri*, ventral view; *p*, pericardium.

Carefully removing the rectum and opening the ventral wall of this common reservoir the latter will be found to terminate posteriorly in two triangular diverticula, each of which communicates by means of a narrow slit immediately behind the transverse blood sinus (sinus transversus) with a dorsal disk-like cavity in contact with the ventral pericardial wall (Fig. 2). From the antero-lateral border of each of these last-named spaces a slender tube arises and passing outward plunges beneath the afferent branchial sinus and then curving backward gradually approaches the surface of the body and opens through the renal papilla. The position of the external excretory opening is, unlike that of the genital papilla, remarkably constant in position. In one hundred specimens examined on this point the excretory pores were, with a very few slight exceptions, situated opposite the last gill.

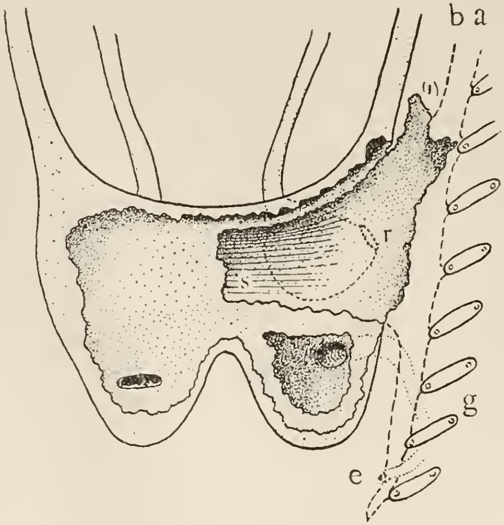


Fig. 2. Posterior part of kidney of *C. stelleri*. The ventral wall has been removed and a portion of the dorsal in order to show the transverse sinus (*s*) and the exit of the ureter; *ba*, branchial artery; *e*, outer kidney opening; *g*, gill; *r*, renal pericardial opening; genital opening opposite eighth gill.

Connective tissue cells laden with concrements, such as were described by Brock ('83) for several molluscs, are present in *Cryptochiton* in great abundance. In all specimens, but notably those above 10 cm. in length they form great accumulations in the foot and mantle in the vicinity of the mantle furrow and give the tissue the appearance of having been charged with light yellow sand which cuts with a distinctly gritty sound. Cells of this character are distributed to a greater or less extent throughout the entire foot and mantle but sooner or later they make their way to the main accumulations by means of amœboid movements and possibly through the agency of the blood stream. In the young of several species of chitons (less than 1 mm. in length) these concrement bearing cells may be seen to originate from the mesenchyme elements that also form the connective tissue, blood and apparently some of the muscle elements. The concrements arise in the form of one or two small refringent granules in each cell and gradually increasing in size and usually in number sooner or later almost completely fill the cell which during this period usually takes up a position near some blood sinus. In the description of the circulatory system attention is called to the fact that a large blood sinus (lateral sinus) with numerous branches penetrates these "Granulazellen" but the relation of the two remains uncertain. Sections not infrequently give the impression that these cells passing into the sinus disintegrate after which the resulting products may be taken up by the kidney but there is no definite assurance that such is the case.

There are only two papers in which a serious attempt has been made to trace the circulation of the blood in the chitons. The first by Middendorff is very incomplete and in many respects incorrect; while the second by Plate is much more detailed yet in certain fundamental particulars, it is not in accord with the results set forth in the following paragraphs.

As we know from the work of Middendorff the heart in this species is essentially the same as in other chitons consisting as it does of a median ventricle and the lateral auricles. This author however made the mistake of claiming that these latter chambers end blindly behind (for they are united in the usual fashion) and that the ventricle gives off numerous small branches in the median ventral line to the rectum and laterally to the mantle edge and last valve of the shell. The supposed openings in this region are merely the depressions marking the attachment of delicate muscle fibers (trabeculæ carneæ) that span the cavity of the ventricle. In the auricles the same depressions appear but as injections clearly show the only blood, besides that from the efferent branchial sinus, that enters these chambers comes from the mantle at the extreme posterior end of the animal through two small vessels close to the mid-line behind and in certain cases even these openings appear to be absent.

The relations of the aorta to the heart and head cavity and the origin of the genital arteries Middendorff correctly determined but the account of the last-named vessels is somewhat obscure. A careful examination shows that they course between the folds of the inner wall of the gland very much as in *Acanthopleura echinata* (Plate) and after branching repeatedly (and often not dichotomously) become lost among the developing sex products.

Concerning the course of the blood from the gonad I have nothing to add to Plate's account of *Acanthopleura echinata* except that in *Cryptochiton* a relatively large quantity leaves from the neighborhood of the gonoducts and enters the extensive sheets of kidney tissue adjoining the front end of the pericardium. From here very little blood appears to go into the visceral cavity but penetrating the spongy tissue of what may be termed the lateral space (Seitenlückenraum, Midd.; Lateralkammer, Plate) is poured into the lateral sinus (Seitenarterie, Midd.).

The dorsal arteries, lateral vessels between the II–V valves, supply some of the shell muscles in the fashion accurately described by Plate. Owing probably to the greater size of the mantle in *Cryptochiton* the intersegmental arteries are more numerous and of larger size than in other species of chitons. They arise sometimes singly (dividing almost at once) though usually in pairs from the dorsal side of the aorta between each valve and as well defined vessels may be traced for considerable distances. Usually these vessels do not extend far into a neighboring "segment" but in all of the specimens carefully examined on this point the vessels between segment V–VI are invariably of large size and extending latterly and posteriorly supply the greater part of the hinder portions of the mantle. The blood returning from the pallium pours into the lateral sinus.

Middendorff (p. 70) states that these small vessels (called by him Mantelarterien) appear to end blindly in the leathery substance of the mantle and on the other hand to open into the lateral sinus and the branchial artery. Plate also makes the statement that in *Acanthopleura echinata* most if not all of the blood from the mantle passes into both the branchial artery and the branchial vein. In *Cryptochiton* the vessels from the mantle are of comparatively large size and may be followed without difficulty into the lateral sinus. Injections of the branchial artery and the branchial vein give no indication that they receive any blood directly from the mantle.

The marginal vessel (Randgefäss) imbedded in the mantle to the outside of the mantle furrow, a position corresponding to the lateral fold (Lateralfalte) of other chitons, is but a system of irregular sinuses supplied by vessels from the intersegmental artery. The blood returning from it passes, together with other blood from the mantle, into the lateral sinus.

In *Cryptochiton* the course of the blood leaving the head cavity is considerably different from that in any other chiton hitherto described. According to several investigators the blood leaves the head sinus by the following vessels: the visceral artery, the lateral sinus of the foot, vessels passing into the snout (Mundscheibe) and by means of canals surrounding the pedal and pallial nerves. In *Cryptochiton* no blood makes its direct exit by either the pedal or pallial neural sinuses and the course of the blood passing into the snout is considerably different from that described by Plate for *Acanthopleura echinata*, and furthermore there is a pair of pallial arteries.

The head cavity in the chitons is a comparatively narrow space surrounding the buccal mass and separated from the visceral cavity by a septum. Among the more important arteries leading out from it are two of large size which spring from the postero-lateral borders of the snout (Fig. 3). Almost immediately each divides, the inner branch, the pedal sinus, passing into the foot, the outer canal, the pallial artery, following along the mantle cavity in close proximity to the pallial or lateral nerve. The pallial sinus shortly after its origin gives off a branch which ramifies throughout the tissue of the proboscis supplying with its fellow all of the region behind the mouth. It is also in communication with an extensive sinus encircling the snout as will be described presently. By means of injections each pallial sinus may be followed into the region of the reproductive opening where, after having gradually diminished in size, it vanishes completely. Delicate branches supply the tissue of the immediate region, some going to the lateral nerve another portion to the tissue of the lateral space and it is possible that another quantity though very small may enter the adjacent regions of the mantle.

Middendorff (pp. 69, 70) speaks incorrectly of two pairs of vessels which arise from the head cavity and passing in close proximity to the mantle furrow open into the sinus transversus (arcus arteriosus). In *Cryptochiton* the only vessel which holds such a position posteriorly is the lateral sinus and this as is well known has no connection whatever with the head cavity, and on the other hand the pallial artery connects with the head cavity but does not communicate with the transverse sinus.

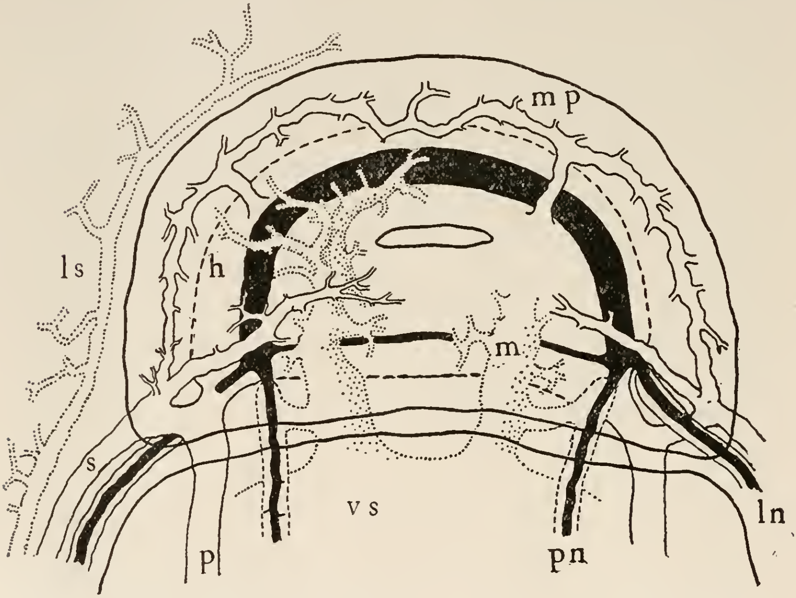


Fig. 3. Diagram illustrating the circulation of snout and certain vessels leaving the head cavity in *C. stelleri*. *h*, head cavity; *ln*, lateral nerve sinus; *ls*, lateral sinus; *m*, median proboscis protractor; *mp*, marginal proboscis protractor; *p*, pedal sinus; *pn*, pedal nerve sinus; *s*, pallial sinus: *vs*, visceral cavity; nervous system, black.

Heretofore it has been held that the sinuses surrounding the pallial cords conduct a considerable amount of blood from the head cavity into the mantle but in not less than 50 specimens of *Cryptochiton* injections from the head sinus failed to penetrate these canals and dissections show that at or near the point of union of the pallial and pedal cords a connective tissue and muscular septum (Fig. 3) completely separates the nerve sinuses from the head cavity. However a short distance behind this point and throughout the greater part of the succeeding portions of the mantle furrow there are frequent communications between the pallial and nerve sinus so that this latter space and adjacent regions are probably filled in life with comparatively pure blood.

In *Cryptochiton* another part of the blood passing out of the head cavity enters the proboscis or snout chiefly by means of two large vessels located on each side of the mid-line about level with the mouth (Fig. 3). Almost immediately these break up into two or three branches that pass outward to the margin of the proboscis where they become continuous with an extensive system of spaces forming a semicircular vessel (*mp*) coextensive with the margin of the snout. From this marginal sinus smaller vessels pass inward and furnish fresh blood to the proboscis tissue, while another supply of arterial blood, much smaller in amount, passes into the snout through sinuses surrounding the nerves. As the figures indicate no part of the blood from any of these sources passes into the tissue of the foot, at least not in any appreciable quantity, but is conveyed along the region of the mantle furrow by means of the relatively large pallial sinus leaving the proboscis at its outer posterior angle, or it may be passed into the visceral cavity as described presently.

Middendorff figures (*f′*, Fig. 2, Pl. VIII.) a small curved canal which surrounds the snout anteriorly and states (p. 72) that it lies between the external and internal oral sphincter. Owing to the fact that in one specimen he was able to force the injection mass from the branchial artery into this vessel he considered it to be the anterior union of the branchial arteries but as these latter vessels certainly do not extend much farther forward than the most anterior gill and have no direct connection with the proboscis, I am of the opinion that the connections as described are incorrect and that the marginal vessel is the same one shown in Fig. 3.

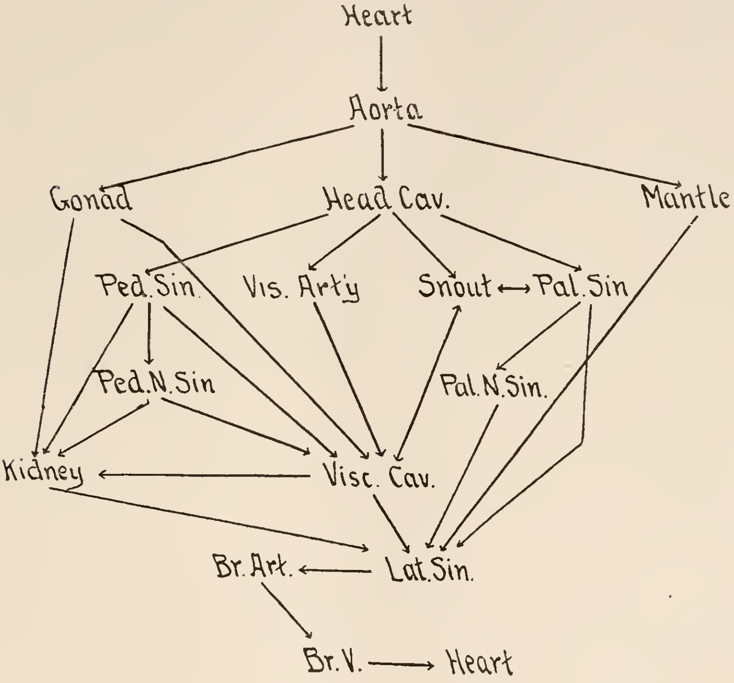
Not only are the margins of the proboscis capable of considerable expansion owing to the influence of an abundant blood supply but as may be seen in living specimens the more central portions bounding the mouth are likewise subject to great distension. This is produced by a system of vessels present also in *Acanthopleura echinata* according to Plate though their connections are considerably different. From the extreme anterior and ventral part of the visceral cavity they arise as two relatively very wide canals one on either side of the mid-line. Coursing almost directly forward they enter the proboscis and giving off vessels into the more superficial portions encircle the mouth almost to the mid-line in front. Upon the contraction of some of the more anterior pedal muscles the visceral cavity is decreased in volume and a portion of the blood coursing through spaces in the region of the stomach is forced into the proboscis resulting within a short time in a marked increase in size and roundness of its central portions. In recently killed specimens pressure on the front part of the foot produces this phenomenon.

From injections it appears very probable that a considerable portion of the venous blood from the snout, when this is not protruded, passes into the visceral cavity by means of these protractor sinuses. The marginal portions of the proboscis and possibly the more central regions also are relieved of a portion, seemingly small, of their blood supply by means of several highly branched vessels emptying into the lateral sinus external to the snout.

In *Acanthopleura echinata* according to Plate the foot is provided with a median sinus which anteriorly connects with two vessels surrounding the mouth and corresponding to the median proboscis protractors of *Cryptochiton.* Were these latter vessels in *Cryptochiton* to be separated with a portion of the visceral cavity and fashioned into a median pedal sinus the relations would be essentially the same as in the above-named species. However the course of the blood leaving the median sinus is not by the usual exit from the visceral cavity for Plate states that it passes into the branchial artery.

The blood enters the foot by the two pedal sinuses, whose relations are already known, and leaves by two routes — into the visceral cavity or along the transverse pedal muscles to the kidney. As has been shown the contraction of some of the anterior pedal muscles may drive a quantity of blood into the proboscis, thus causing its protrusion, and it is reasonable to suppose that according to the state of contraction of the pedal muscles generally the blood pressure of the visceral cavity may vary, causing a flow of blood from the foot into the cavity or in a reverse direction. Injections show that in dead or flaccid animals the line of least resistance is from the pedal sinuses laterally into the kidney.

As with the pedal nerve sinus a well-defined septum destroys any communication with the head cavity (Fig. 3) but throughout its entire course it is in frequent communication with the pedal sinus which supplies it and the adjacent regions with aerated blood.



In all of the specimens carefully examined a connection exists between the pedal nerve sinus and the bases of the median protractor sinus (*m*) so that blood may pass from one to the other. Throughout its greater extent the pedal nerve sinus receives its blood from the pedal sinus and it is difficult to understand this peculiar anterior connection unless it is some device to aid in the protraction of the anterior margin of the foot.

Middendorff correctly describes the visceral artery which springs from the head cavity and surrounding the radula sac supplies the alimentary canal and liver. The blood passing from the capillaries pours into the visceral cavity and joining that from the foot, proboscis and gonad proceeds through the meshes of the kidney to enter the lateral sinus.

The lateral sinus in *Cryptochiton* is a relatively large canal surrounded by connective tissue and muscle fibers in whose wide meshes are vast numbers of "granulazellen." Anteriorily it arises near the mid-line in the furrow surrounding the snout from which it receives a few small vessels while numerous others of large size drain the blood from the mantle. Coursing backward and constantly increasing in size it continues to receive vessels from the mantle and finally in the vicinity of the reproductive opening it unites with the branchial artery.

The accompanying diagram will serve to illustrate the course taken by the blood in passing through the body, at least it represents the main canals. It will be seen that the well-developed lateral sinus plays an important part, acting as a collector for the blood from all parts of the body and possibly its surrounding walls serve to remove certain wastes before being passed to the branchial artery.

The relations of the branchial artery and the branchial vein to the gills and the connections of the latter vessel to the heart are already well known and require no further comment.

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ON THE HETERONEREIS STAGE OF NEREIS KOBIENSIS McINTOSH.[1]

AARON L. TREADWELL.

Among a collection of polychætous annelids made by the U. S. F. C. steamer Albatross in the Hawaiian Islands in 1902, and sent me for study, were large numbers of a heteronereid which by means of the general character of the head, the presence of a peculiar hook-shaped seta in the anterior parapodia, and especially from the form of the paragnathi, I have identified as the above-mentioned species.

Externally the bodies of both male and female individuals show very clearly the distinction between "modified" posterior, and "unmodified" anterior somites characteristic of this sexual phase of *Nereis*, the modifications following in general the usual direction, *i. e.*, a broadening and flattening of all parapodial lobes and a replacement of the ordinary form of seta by one with a very broad, flat, terminal joint. A constant external sex difference is found in the male in the dorsal cirri of somites 2–7. Beginning with somite 2 the dorsal cirrus is larger than in the female, and on successive somites as far as the seventh there is a gradual increase in the size of this organ, until on the seventh it is very prominent, composed of a thick, cylindrical basal portion; with a broad flattened tip, ending in an acute point. This condition is similar to that of the form described by Verrill under the name of *Nectonereis*.[1] On the eighth somite there is an abrupt change to the ordinary form of cirrus.

Internally, extensive modifications appear. On a surface view of a mature female, one sees that the whole body, anterior, "unmodified" as well as posterior "modified" region is crowded with eggs, seeming not to be isolated in somites, but packed together in a continuous cavity. That the transverse septæ are actually lost is indicated further by the fact that by gently pressing on the surface of the body wall, eggs may be moved from one somite to another or through several somites.

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[1] Published by permission of Hon. George M. Bowers, United States Commissioner of Fisheries.

[2] Verrill, A. E., "Invertebrates of Vineyard Sound," Bull. U. S. F. C., p. 591, 1872.

Dissection and the study of transverse sections confirm these conclusions. By the loss of the transverse septæ the whole interior of the body has been transformed into a continuous thin-walled sac, filled with ova from one end to the other. No trace of ovaries could be found, and, owing to the poor preservation of the material nothing definite can be said concerning the histology of the internal organs, but there is every indication of a considerable amount of degeneration. While the pharynx retains its normal condition the intestine appears on gross dissection as a mere thread, and anteriorly, the thin transparent body wall, lacking most of its usual muscle layers, is very noticeable.

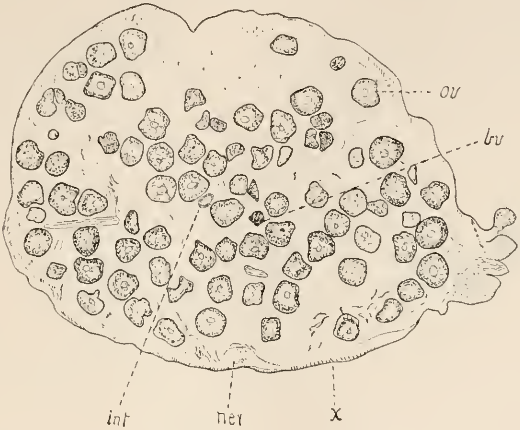

FIG. 1. Section through anterior region of body of female. × 24. *ov*, ova ; *bv*, blood vessel ; *int*, intestine ; *ner*, ventral nerve cord.

Fig. 1 is from a transverse section through the anterior end of the body, thus through the "unmodified" region. The body wall, with the magnification employed, appears as a mere line, with the thin layer of longitudinal muscle fibers showing as rows of dots just inside it. These muscle fibers are more or less broken away from the rest of the wall, but I cannot tell how much of this is due to actual degeneration, and how much to defective preservation and faulty microtome technique. A small break in the dorsal wall is apparently due to the latter. The intestine, *int.*, Fig. 1, shows in the section as a minute tube, in whose walls no definite cellular structure could be seen. In many sections I could find no trace of the intestine. While this may possibly be due to the technique, I do not think that they could have dropped out of so many sections, and believe that there was an actual disappearance. Such a condition with respect to the intestine is not unusual among annelids at the breeding season.

Ventrally is the section of the nerve cord, and on the right a part of the parapodium is seen. The parapodial muscle bands are very feebly developed.

As shown in Fig. 1 large numbers of ova are present in the body cavity. These fill the whole cavity, extending forward into the head, so that, as is shown in Fig. 5 a section through the eye shows at the same time an ovum, contained in a cavity between the muscles of the head. That this condition is usual can be seen by careful surface examination of the entire annelid. The ova appear to have a very dense outer layer with vacuolated interior containing a nucleus. As it is impossible to tell how much of this apparent structure is an artefact, I have represented it only in a very diagrammatic fashion in Fig. 1, while in Figs. 2 and 5 I have drawn merely the outlines of the ova.

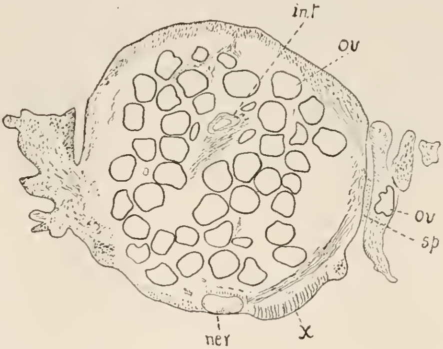

FIG. 2. Section through posterior region of same individual as in Fig. 1. × 24. *sp*, spermatozoa adhering to surface, other letters as before.

Scattered among the ova are many sections of connective tissue and blood vessels, *bv*, Fig. 1, indicating that loose strands of this tissue, carrying vessels, still persist.

In the posterior portion of the body, the so-called "modified" region, similar conditions hold with respect to the septæ and intestine, but the body wall is very noticeably thicker, its muscle layers being very well developed. This is easily seen by reference to Fig. 2 which is a section through a posterior somite of the annelid drawn in Fig. 1. The longitudinal band of muscle fibers is much more strongly developed, and the oblique fibers, especially those connected with the parapodia are vastly stronger than those in the anterior somites. The difference in thickness of the body wall is especially well seen in a comparison of Fig. 3, an enlarged camera drawing of the body wall at *X* in Fig. 1, with Fig. 4, a similar drawing of *X* of Fig. 2. The dermal layer is much thicker, and the longitudinal band is relatively enormously developed. It will be understood, of course, that the thickness of the muscle band is indicated by the entire diameter of the figure. Owing to the irregular arrangement of the fibers no one of them extended through the bundle in a straight enough fashion to appear entire in a single section. It is evident further that the arrangement in Fig. 4 is more nearly a normal one than in Fig. 3, so that the condition of the latter must be regarded as due to degeneration.

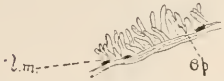

FIG. 3. Detail of ventral wall at *X* in Fig. 1. × 280. *lm*, longitudinal muscle fibers seen in section; *ep*, epidermis.

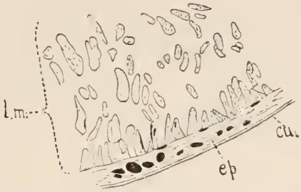

FIG. 4. Detail of ventral wall taken from the point 4 in Fig. 2. × 280. *cut*, cuticle.

On either side Fig. 2 shows a section of a parapodium, that on the right containing an ovum in its cavity.

Similar conditions hold in the case of the male heteronereid. The anterior "unmodified" portion of the body is more or less degenerate as regards its body wall, muscles and intestine, while the posterior "modified" region shows at least no degeneration of its muscle structure. I am inclined to believe there has been a hypertrophy of these organs, but non-sexual individuals are not available for comparison. Spermatozoa are found all through the body, from the prostomium backward. At no place, however, does the intestine appear as degenerate as in the female. This is especially true of the posterior region, where the intestine is large, filling nearly as much of the cœlom as it does in other annelids.

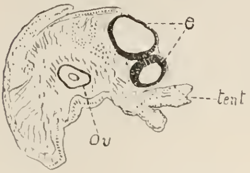

Fig. 5. Section through head of same individual. × 24. *e*, eye; *tent*, tentacle.

If the loss of the intestine has arisen in the female through mechanical reasons, connected in any way with the accumulation of large numbers of ova around it, it is easy to see why the loss should be greater in the female than in the male. Though present in large numbers, the spermatozoa would on account of their small size, occupy much less room and exert much less pressure on surrounding organs, than would the ova.

Many individuals contained no sex cells, apparently having discharged them into the water. That the sea water must have contained considerable numbers of sex cells is shown by the fact that numbers of spermatozoa are found in the sections, adhering to the body of the female. Fig. 2 represents a number of sperm adhering to the outside of a lobe of the parapodium.

As shown above, no sex organs were found. It would be of a good deal of interest to know whether they were originally present throughout the body, or are confined to the posterior "modified" end. The latter condition is much the more probable. Apparently in this annelid there is, in the heteronereis phase, a considerable degeneration of the anterior "unmodified" region, the degeneration involving the internal organs and parapodial muscles, though not especially involving the external organs. Into this thin-walled sack thus formed pass the sex-cells, which are now carried in it (as well as in the posterior portions of the body) while the posterior parapodia retain their normal muscular development and function as the main locomotor organs of the animal. Whether the animal goes to pieces after the sex cells are thrown off, or whether it subsequently regains its normal condition, cannot be determined by the material at my disposal.

Vassar College, March 3, 1905.

THE REPAIR AND REBUILDING OF THE LARVAL CASE OF PLATYPHYLAX DESIGNATUS WALK. (PHRYGANID).

WM. S. MARSHALL AND C. T. VORHIES.

The larvæ of *Platyphylax designatus*[1] are found abundantly in a group of springs in the vicinity of Madison, and, being easily obtained at any time during the year, have been collected for work in the laboratory. Experimenting with a few larvæ which we kept in small aquaria, we noticed that those which had been removed from their cases constructed new ones and also that injuries to the cases, by removal of some part, were generally repaired in a short time by the larvæ. This led us to carry on a number of experiments to determine how far injured shells would be repaired by the larvæ, and, if certain parts of the cases were more frequently and readily rebuilt than others.

The springs in which these Phryganid larvæ live remain open all the year; this was a great advantage to us in that material could be readily obtained throughout the winter. The water in the springs is very cold during the summer as well as in the winter months and the larvæ, being accustomed to very cold water, would not live, during the summer, in aquaria kept in the laboratory or out of doors. We found it advisable to carry on our experiments during cold weather, at which time it was easy to keep the aquaria in cool places, bringing them into the laboratory only when our specimens were examined. In the autumn and in the spring, just before or after freezing began or ceased, we found the most convenient times for our work, as at these periods of the year the aquaria could be kept on a ledge outside of the laboratory window.

The cases of the larvæ of *Platyphylax designatus* are constructed, with few exceptions, from the sand which covers the bottom of the springs in which they live. Irregularities in the size of the sand grains used are sometimes observed, but there is, in general, a regular arrangement; the posterior, oldest, part of the case is made entirely of small grains, the anterior, newest, part of the shell of much larger and more irregular grains; between the two ends there is a gradual increase in the size of the sand grains used. As the larvæ increase in size the shell is made wider and larger grains are used in its construction; after building is completed, or nearly so, there are often two or three very large pieces placed near the anterior end. When the larvæ begin to prepare for pupation nearly all the pieces added are of greater size than any of the others, and, finally, when the case is closed, the anterior fourth or third is nearly covered with these large grains, which are so marked and constant that by their presence one can easily tell a larval from a pupal case. Often just before pupation a larva would close the posterior end of its case by the addition of a single large sand grain over the opening at this end. After closing the shell for pupation a small opening was always left at the anterior end. This was found between two of the large sand grains, the space between which was filled with a layer of the secretion from the spinning glands, and in this the opening was found.

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[1] The identification of the species was made by Mr. C. Betten.

Two different kinds of plant seeds were sometimes used in the construction of the cases and these, with the rare use of pieces of snail shells, composed with the sand grains, the materials of construction. In many instances when the seeds had been used in building a case but one or two of them were used; cases were, however, often found in which were a dozen or more of the seeds and often the two kinds were found in the same case. We had several larvæ with these cases living for a few days in the laboratory where the water remained warm and this sometimes resulted in the germination of the seeds. When this happened the larvæ presented a curious appearance, their cases having a few young plants, one quarter to one half an inch in length, growing on them.

The experiments we made were upon the construction of new cases by larvæ from which the original cases had been removed, and upon the repair of cases after certain parts had been cut out. To facilitate the work and make it possible for us to tell exactly how much new shell had been added, we placed in our aquaria, not the brownish sand from the springs in which the larvæ lived, but crushed red sandstone. When a larva rebuilt the part of the shell we had removed it was easy to see just how much new material had been added.

The construction of a new case is easily observed by removing larvæ from their cases and placing them in aquaria at the bottom of which is some sand. The larvæ appear at first much distressed and wander around in the aquaria attacking each other, if a number are in the same dish, but never, so far as we have observed, injuring one another. From the fact that the caseless larvæ always attack each other it is much better to place but a single one in an aquarium. Many will remain for one or two days without attempting the construction of a new case while others will begin building within an hour, or in a few instances, in even less time. When a larva begins the construction of a new case it first, with its mandibles, gathers a few of the larger sand grains and cements them together with the secretion from the spinning glands. This done, the larva gathers other grains from a distance carrying them in its mandibles to the pile already formed, and, as each is brought, it is cemented to the others. In this way, in the course of from two to four hours, a large pile of sand grains is made, each grain of which is cemented to the others, forming all together a loose mass nearly as large as the larva itself. It is now somewhat surprising to notice that the larva, in beginning to construct its shell, does not, apparently, use this pile at all but begins its case at one side and adjacent to it, and there, from smaller grains than those in the pile, the new case is built. The first process in the actual construction we did not see but the larva soon has a narrow band of case around its body and to this, at the anterior end, new grains are continually added. As the new case increases in length, the diameter is slightly enlarged although the new cases do not show as much difference in width at the two ends as did the old shell. When the case has reached the necessary length the larva turns in it and begins to pull down the posterior margin, finally making the opening at this end smaller than the diameter of the shell. The larva again assumes its original position and finally builds a slight dorsal hood at the anterior end. During the construction of the case the original pile of sand grains has been attached to its posterior end and may be dragged around by the larva; it is however finally cut off and remains at the bottom of the aquarium. Whether or not the construction is similar in the natural habitat of the larvæ we do not know.

CASES CUT LONGITUDINALLY FROM END TO END.

Without removing the larvæ the cases were cut with scissors from end to end and on different surfaces. It was necessary to cut the cases slowly avoiding the largest sand grains and taking care not to injure the larvæ.

1. The cases of three larvæ were cut, one dorsally, one ventrally, and the third along the lateral surface. The following day the laterally cut one had glued the cut surfaces together in the anterior region; the other two had not been repaired.

2. The experiment was repeated with three more larvæ and at the end of twenty-four hours all had glued, anteriorly, the cut edges together for one third the distance.

3. The case was cut ventrally in one larva and on the following day it had been repaired the same as above.

4. A larva with case cut dorsally gave the same result.

5. On the twenty-sixth of the month a case was cut dorsally and the larva removed from it; the following day it had returned to its case and cemented the edges at the anterior end for about one quarter of the distance. Twenty-eighth, the larva had cut out a notch at the anterior end, at terminus of original cut, and built in with new sand. Twenty-ninth, had added a few sand grains to posterior edge of shell. First of following month, had added a band, two grains wide, at the anterior margin.

6. On the twenty-fifth a case was cut dorsally which by the following day had been repaired at the anterior end for about one third its length. Twenty-eighth, case cut again in the same plate. Twenty-ninth, no repair. Thirtieth, edges glued a little at anterior end and two rows of sand grains added to this margin. Thirty-first, same. First, cut again. Second, glued anterior third of distance and added from four to five more rows of sand at this end; cut again in same place. Third, again glued at

anterior end and more sand added, making a total of 2.5 mm. Fourth, cut again. Fifth, had cemented at anterior part; cut again. Sixth, cemented as before; cut again. Seventh, eighth, ninth and tenth, cementing and cutting repeated each day. Eleventh, cut edges at anterior end cemented as before and a little more new sand added to this end.

7. Six cases were cut, two dorsally, two ventrally, and two laterally. The time during which these experiments were carried on was at least two weeks in each instance, giving sufficient time for full action on the part of each larva.

Of the two cut dorsally one ignored the cut entirely but built on a ring of new sand at the anterior end after eight days had elapsed. The other at once cemented the cut edges for a distance of 2 mm. at the anterior end, then, for a period of nine days, did nothing further. Repetition of the cutting had no other effect than to cause, within a short time, the larva to glue the edges for a short distance at the anterior end.

The two cut ventrally took much more notice of the injury to the case than the preceding. One of these closed the cut half way down from the anterior end and at the end of two days had completely glued the cut edges together. Repeated cutting caused, with one exception, a partial repair within twenty-four hours. After five such repetitions an additional day was allowed for repair, at the end of which time, the cut edges were completely glued. The second had at the end of twenty-four hours cemented the cut at the anterior end for a distance of 2 mm.; then for eight days it did no further repairing. The cut was then reopened and at the end of twenty-four hours was again partially closed. The next day the larva had turned in its case and was working at the posterior end and on the following day had completely cemented the cut edges.

The first of the two laterally cut specimens would always close the cut for about one third the distance at the anterior end, and once, when allowed four days, completely repaired the injury. Twice a new ring was added at the anterior end instead of the usual repairing. The second of these larvæ did nothing for four days. It then closed the cut for a distance of 2 mm. at the anterior end and for four additional days made no further repair.

The cut was now well opened daily for seven days. On each of the first two days a distance of 1 mm. at the anterior end was repaired, on the third and on the fourth days 2 mm., and on the fifth and sixth days a distance of 4 mm. was repaired. The repairing on the sixth day was very poorly and loosely done and, no repair being made on the next, the seventh day, the experiment was abandoned.

The cutting of the cases from end to end causes no inconvenience to the larva and no exposure of its body. In all examples cut it was difficult to see, without a fairly close examination, that a cut had been made; the firmness of the case causes the cut edges to either touch along their entire distance or, in many instances, to slightly overlap. The repair was always begun at the anterior end, the larvæ paying but slight attention to the opposite end although, in a few cases, this was also repaired and it may be, that if the cut cases had been in every instance left unmolested for several days the ultimate result would, in all experiments, have been a completely repaired case. The distance of the repair at the anterior end represents the distance which can be readily reached by the larva without turning in its case.

Notches cut in Case at Anterior End.

These notches were cut in from the anterior margin of the case and on its different surfaces; the cuts were in general equal in depth to one fourth or one fifth the length of the shell. While not always of the same size and shape the piece removed was generally very nearly 3 mm. in length and 2 mm. in width at its base, the base of the triangular piece cut out being always along the anterior margin of the case.

1. Twenty-ninth, lateral notch cut; in two days this was half filled with new sand grains; the next six days no change. In thirteen days the notch had been completely filled in and a few grains added to the anterior edge.

2. Dorsal notch cut, was completely repaired in two days.

3. Dorsal notch cut and partially repaired by following day; in building a small opening was left by the larva at the apex of the notch and this was never repaired. In two days the anterior margin was built even to rest of case.

4. Dorsal notch cut in case and it was completely filled the following day; this was repeated seven more times and each time the notch was filled in twenty-four hours; the ninth time the repair took two days. The larvæ now added a narrow band to the anterior edge of the case; this was removed and rebuilt three times in as many days.

5. Dorsal notch cut and repaired seven times daily with one exception, in which the period of repair extended for two days.

6. Ventral notch cut six times and repaired five times, each at end of twenty-four hours, and once after interval of two days.

7. Two cases, in each of which a dorsal notch was cut, were repaired by the following day.

8. Dorsal notch cut but not repaired for three days.

9. Lateral notch cut and repaired in three days.

10. Two cases notched, one dorsal and one ventral, were both repaired in twenty-four hours, but in each instance a small opening was left at apex of notch which was never repaired.

Any portion of the case which has been removed from the anterior part, provided the piece removed includes part of the margin, was repaired in a short time. If the piece removed was taken from the shell at some place away from the margin, repair is neglected and the opening allowed to remain. In two of the experiments recorded it will be noted that in filling in the notch a small part was left open and this was never repaired. Some larvæ not recorded had holes cut in their shells and after a lapse of several days the openings were still unrepaired. A larva can easily fill in a notch 2 × 3 mm. in twenty-four hours.

Case Cut Transversely into Two Pieces.

In the following experiments the cases were cut transversely into two pieces, which were as nearly equal as we could make them without an actual measurement. The larvæ were then replaced in the aquaria with both pieces of the case still on their bodies.

1. On the first of the month a case was cut and one piece, 6 mm. in length removed. Second, at 8 a. m., the new part added to old piece left on the larva was nearly equal to the part removed, and at 4:30 p. m. it was of the same length. Third,

the new part was now 8 mm. long, or 2 mm. longer than the part removed, and it was also somewhat wider. Fourth, new piece 10 mm. in length. Seventh, larva had added 2 mm. more and closed the shell for pupation.

2. Fourteenth, case 11 mm. in length cut and both pieces left on larva. Fifteenth, the larva had thrown off the posterior piece and built 2.5 mm. to anterior end of piece (anterior) which it had retained; total length 7 mm. Sixteenth, length of shell had been increased to 11 mm., the original length.

3. Fourteenth, case 10 mm. long was cut and both pieces left on the larva. Fifteenth, the posterior piece had been thrown off and 3.5 mm. built on to anterior end of remaining piece. Sixteenth, enough had been added to make entire length of case 10 mm.; equal to the original.

4. Second, a case 11 mm. long was cut and on the following day the posterior half had been thrown off and 4.5 mm. added to anterior end of the piece retained by the larva. During the day the larva reversed itself in the case and turned in the posterior edge; total length 9 mm. Fourth, removed the new anterior part which the larva had built. Fifth, had again added 4.5 mm. to old part of case. Eleventh, abandoned old shell and built a new one which, by the fourteenth, was 9 mm. long.

5. Second, a shell 9 mm. in length was cut and by the following day enough had been added to the anterior piece of the old case to make it 10 mm. long. Fourth, cut between old (posterior) and new (anterior) parts. Fifth, had thrown off the old (posterior) piece and added 3 mm. to other part. Seventh, case had total length of 9 mm. Fourteenth, total length 10 mm.

6. Fourteenth, cut a shell 10 mm. long; the same day the larva threw off the posterior piece and added 2 mm. to the anterior end of the piece. Sixteenth, length of new part only 3 mm. and on the next day had increased to 3.5 mm.; total length of case 9 mm.

7. Thirty-first, case 11 mm. in length was cut and on the following day the posterior piece had been thrown off and 4.5 mm. added to anterior end of the piece retained by the larva. Third, case was cut between the new and the old parts. Fourth, old part (posterior piece) had been thrown off and enough added to

other piece to make total length of shell 10 mm. Sixth, the posterior end was turned, total length of shell 10.5 mm.

8. Second, shell 9 mm. long was cut: on the following day the posterior piece had been thrown off and 3 mm. added to anterior end of remaining part. Fourth, posterior end had been turned; 3 mm. of the new shell removed. Fifth, 1.5 mm. added. Seventh, total length of shell now 7 mm; following day the larva was dead.

9. A shell 12 mm. long was cut and on the following day the two pieces were glued together and a few new sand grains added to the anterior end.

10. A shell 12 mm. in length was cut and on the following day the posterior part had been thrown off and 5 mm. added to anterior end of remaining part. After an interval of one more day 7.5 mm. of new shell had been added; total length 12.5 mm.

11. Fourteenth, shell 12 mm. long cut. Fifteenth, posterior part had been thrown off and 2 mm. added to anterior end of remaining part. Sixteenth, 5 mm. more added. Seventeenth, 0.5 mm. added: total length 9.5 mm.

12. Thirty-first, case 11 mm. long was cut. First, posterior part thrown off and 4.5 mm. added to anterior end of other part. Second, no change. Third, cut again at boundary of old and new parts. Fourth, the posterior piece again thrown off and enough added to make total length of case 10 mm. Eighth, posterior end turned, total length of case 10.5 mm.

In but one of these experiments did the larva glue the two cut margins together and when this had been done the case was nearly as good as before cutting, except its greater flexibility and a slight lack of strength. In every other experiment the larvæ threw off one of the pieces, always the posterior, and built on to the anterior margin of the remaining (anterior) piece. The construction of the new part of the case was rapid, averaging nearly 4 mm. the first twenty-four hours; to this more material was added until the case had entirely or nearly reached the original length. One half of the case is not long enough to entirely conceal the body of the larva and the first work is to increase the length of the case sufficiently to do so, after which what we might call the finishing touches, *i. e.*, turning in the posterior margin and building of a hood at the anterior end are finished.

REMOVAL OF PART, OR WHOLE, OF POSTERIOR END.

1. Thirty-first, cut off the posterior end of a case and by the following day the larva had turned in the edge; cut again. Third, had slightly turned the edge. Fourth, cut 2.5 mm. from posterior end. Fifth, had added 5 mm. to anterior end. Eighth, 2.5 mm. more added. Tenth, posterior edge turned.

2. Small piece cut from the posterior end and by following day the edge had been turned and four grains of sand added at this end; case but slightly smaller than before cutting. Following day the larva was dead.

3. Second, cut piece 4.5 mm. long from posterior end of a case which was 11.5 mm. in length. Third, added 3 mm. to anterior end. Fourth. 1 mm. more had been added. Seventh, 2.5 mm. more added and anterior end closed. Eighth, both ends of case closed and it was glued to a leaf for pupation.

4. Second, cut piece 3.5 mm. from posterior end of a case. Third, 1.5 mm. had been added to anterior end. Fourth, 0.5 mm. more added. Tenth, new part now 3 mm. in length, posterior end not yet turned.

5. Ninth, cut 4 mm. from posterior end of a case and by the following day 3.5 mm. had been added to the anterior end. Fourteenth, a few sand grains added to the posterior end; case closed for pupation.

These few experiments on the posterior end show that the larvæ are loath to work at this end of the case and the additions to the shell were made at the anterior end. As the cases are normally turned in at the posterior end most injuries to this end will result in the larvæ ultimately reversing themselves in the case to re-turn the margin. We watched the larva turn from a reversed to a normal position, the whole process occupying about four minutes. At first the posterior end of the body is protruded from the anterior opening; the head is then bent forward and soon appears at the opening. Next, by a seemingly very strenuous effort, the body is withdrawn into the case and the larva assumes its normal position. During the process the second pair of legs are thrown up into a position dorsal to the thorax and pointing backward. The larva takes a short rest during the reversing process. That the effort is an extremely hard one is evinced by the fact that we have, during our experiments, found larvæ which have been "stuck" in turning and died without being able to completely turn.

Notches Cut at the Posterior End.

1. Four cases were cut, two dorsal and two ventral. The two ventrally and one dorsally notched case were closed the following day, but without the use of sand, the secretion from the spinning glands being used and the cut edges partly drawn together. The fourth specimen did nothing on the first two days; the third day, and, thereafter each day for five days, new grains were added to the anterior end until a new part 4 mm. in length had been built.

Two experiments were made by cutting, day after day, a small piece from the posterior end of the case, thus forcing the larva to add continually to its case if it desired to keep it long enough for its body. It was found that either daily or every two or three days a small portion would be added; if daily, a smaller amount than if the larva waited one or two days before making the repair. No tendency to exhaustion of the silk was noticeable during the three weeks or more that the experiments lasted.

No. 1 built a total length of new case 17.5 mm. in twenty-one days; average 0.83 mm. daily.

No. 2 built a total length of 19.5 mm. in twenty-three days; average 0.84 mm. daily.

With several larvæ the experiment was tried of driving them out of their cases and then placing both larva and case in the same dish to ascertain if they would return to old case. Two larvæ were placed in each dish, a large and a small one, to see whether a larva would occupy a case built by another; with this arrangement there could of course be but one change, that of the smaller larva into the case of the larger. When new cases were constructed these were destroyed leaving only the original ones.

1. Did not return to old case. Built a total length of 32 mm. of new case in the first four days. Rested a day and then added 6 mm. more in two following days. Total length of new case built in seven days 38 mm. Average 5.42 mm. per day.

2. Reëntered case at the anterior end, the regular method, but in reversing stuck fast and died.

3. Returned to old case five times out of seven trials. Built 12.5 mm. in eleven days. Average 1.1 mm. per day.

4. Out of eight trials returned to its old case twice and to that of larger larva once. Built five new cases in thirteen days with a total length of 42 mm. Average 3.23 mm. per day.

5. Did not return to its old case. Out of eight trials lasting thirteen days a new case was built each time but one. Daily average 4 mm.

6. In six trials built a new case each time then occupied case of larger larva twice, the last time adding a new part of smaller diameter to the shell. In twelve days built 42 mm. of new case. Average 3.5 mm. per day.

7. Out of six trials returned to old case five times, building only 5 mm. of new material. Went without case for two days during which time the old case was occupied by another larva. Daily average of new case construction only 0.38 mm.

8. In eight trials a larva returned once to its own case, three times to that of a larger larva, and four times built new cases; 23 mm. of new cases were built in fourteen days. Daily average 1.64 mm.

9. Did not return at all to old case but built, in fourteen days, a total of 65 mm. Average daily construction 4.64 mm.

10. November 7, took a larva out of its case and it immediately crawled in again at the anterior end. Eighth, had assumed proper position in case and added new ring of sand grains at anterior end. Removed again from case but it reëntered as before. On the ninth, tenth and eleventh, the larva was removed from its case but each time time reëntered and added a little new material to the anterior end, 3 mm. in all. Twelfth, removed from case but did not again return and not until the sixteenth was a new case constructed, at which time it was 10 mm. long. Seventeenth, case 15 mm. in length. Eighteenth, case 16.5 mm. long; removed larva from it. Nineteenth, had constructed a new case which was 11 mm. long. Twenty-first, new case had 2 mm. more added; removed the larva. Twenty-second, another new case 7 mm. long had been built; again removed the larva.

Twenty-third, the larva had returned to its original case. During the six days of its greatest activity in building the larva had constructed 36.5 mm. of new case, a daily average of 6.08 mm. The daily average for the entire experiment was 2.25 mm.

Zoölogical Laboratory, University of Wisconsin,
April, 1905.

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A SHORT REMARK UPON W. H. LEWIS' "EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE EYE IN AMPHIBIA."[1]

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In the above-mentioned article, published in Volume III. of the *American Journal of Anatomy* (1904), W. H. Lewis has given us the results of a large number of ingenious experiments on frog-larvæ intended to determine the possible correlations between the development of the optic vesicle and that of the lens.

These experiments have, in the first place, offered a new and, as it seems to me, very convincing proof that there is indeed, as first maintained by Herbst and Spemann, a distinct correlation in the development of these two organs, namely in such a way "*that in normal development the lens is dependent for its origin on the contact influence or stimulus of the optic vesicle on the ectoderm.*"

Furthermore Lewis has, by transplanting the optic vesicle to another part of the larval body, demonstrated "*that the optic vesicle can stimulate a lens to form from various portions of the ectoderm and even from the ectoderm from the abdomen of another species of frog. . . .*" From this fact he has drawn the following conclusion : "There is no predetermined area of the ectoderm which must be stimulated in order that a lens may arise. On the contrary various portions of the skin when stimulated by the contact of the optic vesicle may and do give rise to a lens. Not only will a lens arise from various places on the skin as a result of the contact of the optic vesicle of the same animal, but the optic vesicle of one species may cause a lens to rise from the ectoderm of another species of frog." This latter result of Lewis' experiments is of very great interest, in so far as it gives a new evidence of the *equipotential nature of the ectoderm elements* during a certain early period of development. However in further discussing his grafting-experiments and some other observations made in connection with them Lewis is also led to a criticism of a theory recently advocated by me, *namely that the lens has to be considered as a modified primitive sense organ.* As this criticism seems to be based upon a misconception of my article on this subject[1] I feel induced to write the following remarks in explanation.

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[1] Lewis, W. H., "Experimental studies on the development of the eye in Amphibia. I., On the origin of the lens, Rana palustris," *American Journ. of Anatomy*, III., 1904.

Lewis says on page 535 of his paper: Schaper's theory "will not hold in view of the fact that the ectoderm, taken from over the abdomen of *R. sylvatica* and grafted on over the optic vesicle of *R. palustris* (see experiment XII., 51), did not possess at the time of operation the primitive sense organs and yet it gave rise to a lens. Again it seems unlikely that in several instances in which I have been able to bring about lens formation from a strange ectoderm that the optic vesicle should have in each case come in contact with one of these sense organs. And again in such experiments as IV., in which the optic vesicle has never been in contact with the ectoderm which normally gives rise to a lens there is no trace of a rudimentary lens such as Schaper pictures." From this remark it is obvious that Lewis holds, that according to my opinion the lens should arise in the course of *ontogenetic* development from a *preformed* primitive sense organ of the skin.

In refutation of this, the following must be said. In the first place I have to state that nowhere in my above cited paper have I spoken of a *preformed* primitive sense organ ("Sinnesknospe") as being the *ontogenetic* forerunner of a lens. What I have maintained is, that the structure which arises from the stimulated part of the ectoderm, to form the lens, shows in its development and its primitive histological features a striking resemblance to a primitive sense organ of the skin, and that this resemblance becomes still more striking when, as in my experiments, by atypical development the lens-anlage is retained within the ectoderm. The evidence of this similitude is, as I think, fully demonstrated by the figures 9, 10 and 11 in my paper. Again, I have by no means considered the anlage of the lens within the ectoderm as a regular (*i. e.*, functional) sense organ ("Hautsinnesorgan"), as I have especially emphasized in the statement: Es "liegt uns selbstverständlich der Gedanke fern, unsere Lentoide functionell auf gleiche Stufe mit einer Sinnesknopse stellen zu wollen, . . .". I have always spoken of a *phylogenetic*, never of an ontogenetic deduction of the lens from a primitive sense organ. Finally I have expressed the opinion that the formation of "Sinnesknospen" is one of the most primitive tendencies of all ectodermal elements and that in any part of the ectoderm a bud-organ may arise under adequate stimulus. This being admitted, the conclusion seems to me justified that also the contact stimulus of the optic vesicle upon the ectoderm may awaken these most primitive properties of the ectodermal cells and result in the formation of a structure *morphologically* homologous with a primitive sense organ which in the course of phylogenetic development has gradually entered a new path of differentiation.

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[1] Schaper, A., "Ueber einige Fälle atypischer Linsenentwirklung unter abnormen Bedingungen. Ein Beitrag zur Phylogenie der Linse und zur Mechanik ihrer Entwicklung," *Anatom. Anzeiger*, XXIV., 1904.

Taking all this into consideration, *the absence of preformed primitive sense organs in that part of the skin where the optic vesicle comes in contact with it and where in consequence the lens is formed is by no means an objection against my theory.* When my theory holds that the lens, from a morphological point of view, has to be considered as an ancient sense organ, which arose, it is true, *originally* from a primitive sense organ of the skin ("*Placoden-Organ*" *of Kupffer*), it does not of course imply the necessity that during *ontogenetic* development of later animal forms we should still be able to see the lens evolve *directly* from such an organ. Just as the mode of development of so many other organs, that of the lens also may have undergone, in the course of phylogenetic progress, considerable modifications until the original process of differentiation becomes more or less concealed. Now, if an organ, as in the case of the lens, *still* exhibits in its early ontogenetic features some distinct reminiscences of ancestral characteristics, we are all the more justified in drawing conclusions from such phenomenon in regard to the ancestry of the organ in question.

Hence, my observations upon the development of the amphibian lens (especially under abnormal conditions) having assured me of a close resemblance of this process to the differentiation of a primitive sense organ, I have advocated this fact as a new evidence, in addition to many others, of the originally sensory nature of the crystalline lens.

NOTES ON THE NORTH AMERICAN SPECIES OF BRANCHINECTA AND THEIR HABITATS.

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Three species of *Branchinecta* have been recorded for North America: *B. paludosa* (Müller) Verrill has been reported from Labrador and Greenland. This is an arctic species and is found also in northern Europe and in Siberia. *B. coloradensis* Packard and *B. Lindahli* Packard have been found in the United States.

Of the last two species *B. coloradensis* is the better known. It has been found in Colorado, where it occurs in great numbers, and is recorded for several different localities, all of which, however, are near the center of the state. The original collections were made "near Twin Lakes Creek" south of Leadville, at an elevation of about 3,800 m. (Packard, 1874: 621). Later it was taken near Gray's Peak not far from Georgetown at an altitude of about 3,658 m. and at Weston's Pass southeast of Leadville at an elevation of 3,557 m. (Packard, 1883: 339). After an interval of 21 years it has been recorded recently from Dead Lake in the Pike's Peak region at an elevation of 3,340 m. (Ward, 1904: 139).

B. Lindahli was collected first in a pool at Wallace, Kansas, by Professor Joshua Lindahl, and described and named by Packard (1874: 339–340). Since then, Lafler and Pearse (1898) reported "five or six" specimens from De Witt, Nebraska, and Beardsley (1902: 43) records "one female with eggs" from a temporary pool near Greeley, Colorado. Accordingly this species occurs at a much lower elevation than *B. coloradensis.*

During the summer of 1904 the writer collected 122 adults and many larvæ of *B. coloradensis* from Dead Lake, in the Pike's Peak region. Nine specimens were taken from the same Lake on August 12, 1903.

On October 23, 1904, Professor Aven Nelson collected 16 specimens of *B. Lindahli* at Laramie Hills near Laramie, Wyoming, at an elevation of 2,317 m. These specimens were found "in an eroded limestone bowl in a canyon always dry except after showers. The bowl will hold a half dozen tubs of water and is rarely entirely dry." This collection was sent to Doctor Ward and was identified by the writer. It contained 10 males and 6 females. This record greatly extends not only the known geographical distribution of this species, but also its vertical distribution. It now belongs with *B. coloradensis* and *B. paludosa* to the short list (Zschokke, 1900: 188) of Phyllopods already recorded in alpine situations. This material was carefully measured and compared with the material of *B. coloradensis* and also with the measurements and descriptions of *B. paludosa* as given by Verrill (1870), Packard (1883) and Sars (1896). The results of these measurements are given in the above table:

Species.	*B. coloradensis.*					*B. Lindahli.*					*B paludosa.*		
	Number of Specimens Measured.	Average.	Maximum.	Minimum.	Measurements by Packard.	Number of Specimens Measured.	Average.	Maximum.	Minimum.	Measurements by Packard.	*B artica* Verrill.[1]	*B. grœnlandica* Verrill.[1]	Measurements by Packard.
Length of male	40	19.6	23	16	18	10	13.6	17.5	11.2	8	20	17	15–19[2]
Length of female	45	18.3	20.5	16	17	6	12.8	15.5	10	15	20		12–18
Second antenna of male	40	5.33	9	3.5	7	10	3.87	5	2.26	3			4– 5
First division of second antenna	40	3.344	6	2.1	4	10	2.23	2.92	1.29	1.5	1.66	2.87	2.5–3
Second division of second antenna	40	1.986	3	1.2	3	10	1.64	2.11	.975	1.5	1.29	2.24	1.5–2
Eye of male	40	.715	.869	.650		10	.428	.537	.325		.66		
Eye of female	31	.607	.685	.571		6	.321	.373	.276				
Egg	25	.328	.373	.308		4	.189	.195	.162				
Length of ovisac	45	6.17	8	4	8	6	3.71	5	2	4–5	6.2		4– 5
Caudal appendage of male + setæ	34	1.88	2.27	1.46		10	1.92	2.6	1.62		1.96	1.62	
Caudal appendage of male	35	1.13	1.46	.812	1	10	1.32	1.62	.975	1	.96	.86	1–1.5
Caudal appendage of female + setæ	6	1.80	1.99	1.19		6	1.95	2.27	1.42				
Candal appendage of female	10	1.143	1.4	.65		6	1.115	1.46	.853	2			

[1] Verrill originally thought these distinct species; his measurements are given in these columns.

[2] Sars places the maximum limit at 23 mm. Measurements are all in millimeters.

It is clear from the measurements that *B. coloradensis* and *B.*

paludosa are of about the same size, while *B. Lindahli* is somewhat smaller. The body of *B. coloradensis* is not only larger but more robust than that of *B. Lindahli.* The latter is much more "fairy-like" in appearance, and specimens in formalin are perfectly white or transparent. The color of *B. coloradensis* is much more variable. It has not changed in formalin and varies from creamy white to salmon. In the material collected July 29, 1904, were 26 males and 56 females; the males were light and the females were salmon without a single exception. But of the 14 males and 26 females collected July 12, 1904, there were several exceptions to this color distinction.

Perhaps the most noticeable difference between *B. Lindahli* and *B. coloradensis* is in the size and shape of the eye. (Pl. I.; 13, 14, 15, 16). Packard (1883 : 338) describes the eyes of *B. coloradensis* as "rather larger" than those of *B. paludosa*, and the eyes of *B. Lindahli* as "rather large." A reference to the table shows that the eyes of *B. coloradensis* are much larger than those of *B. Lindahli*, the ratio derived from the average measurements being for the males 1.67 to 1 and for the females 1.89 to 1. It must be understood that, since the writer has only 6 females of *B. Lindahli* the ratio for these may not express as nearly the average as does the ratio for the males. Verrill (1870 : 245) gives 0.66 mm. as the measurement of the eye of the male of *B. paludosa.* This almost equals the smallest measurements of *B. coloradensis*, but is considerably larger than the largest measurements of *B. Lindahli.* In *B. Lindahli* the ocular globe is developed but slightly better on the anterior side than on the posterior side. In *B. coloradensis*, however, the anterior side is developed much more than the posterior, or it may be said that the eye is bent forward abruptly at the place of union between the ocular globe and the peduncle. When viewed from above, these eyes are very distinct from those of *B. Lindahli* and can be distinguished at a glance. Although the eyes of the female are somewhat smaller in each case than those of the males, they are of the same form. Judging from Sars' description (1896 : 44; Pl. VI., Fig. 9; Pl. VII., Figs. 1 and 2) the eyes of *B. paludosa* are more like those of *B. Lindahli* than like those of *B. coloradensis.*

The second antennæ, or claspers, of the males differ rather markedly in these three species. *B. paludosa* is distinct because of the toothed inner margin of the first division, and of the gradually tapering second division which ends in a bluntly rounded point. In both *B. coloradensis* and *B. Lindahli* the distal ends of the second antennæ are turned in rather abruptly (Pl. X., 7, 8; Pl. XI., 23, 24). *B. Lindahli* has, as a rule, shorter claspers than *B. coloradensis.* But this cannot always be depended upon, for the measurements show that it is not an uncommon thing to find *B. Lindahli* with second antennæ longer than those of *B. coloradensis*, even though the body length of the latter exceeds by several millimeters that of the former. There is, however, a considerable difference in the average length of the claspers of these two species. The average measurement of 40 specimens of *B. coloradensis* is 5.33 mm., varying from 3.5 mm. to 9 mm.; while the average of 10 specimens of *B. Lindahli* is 3.87 mm., varying from 2.26 mm. to 5 mm.; the ratio between the averages of *B. coloradensis* and of *B. Lindahli* is 1.37 to 1. Only one individual of *B. coloradensis* had claspers 9 mm. long, and this is probably exceptional, since the next longest measured only 7.2 mm.

The basal division of the claspers of *B. Lindahli* is of about the same thickness as that of *B. coloradensis.* It is somewhat shorter, however, the ratio being 1. to 1.49. The outer margin of the first division of the clasper of *B. Lindahli* has a number of very delicate sensory hairs. Near the base on the inner side there is a raised portion bearing teeth (Pl. X., 7*ta*). Teeth are totally lacking as we approach the distal end. This division of the claspers of *B. coloradensis* is notably different. The toothed area is about half way between the ends on the inner side (Pl. X., 8*ta*). Near the base there is a prominent tubercle of considerable length. This tubercle is characteristic of full grown males. It is not plainly seen unless the clasper is bent forward, when it stands out prominently on the inner side. It is often a third as long as the width of the basal division (Pl. X., 8*t*; Pl. XI., 23).

The second division of the clasper is about the same thickness in the two species. The length is somewhat less in *B. Lindahli* than in *B. coloradensis.* In each species the inturned portion varies greatly in outline, depending on the point of view (Pl. X., 7, 8, 9, 10, 11, 12; Pl. XI., 23, 24). Viewed from the front or back, the tips of *B. Lindahli* are much more gradually pointed than those of *B. coloradensis* (Pl. X., 7, 9, 11), but when viewed from above or below, the reverse is true. This is due to the fact that the already flattened second division of *B. Lindahli* is still more flattened on the outer or lower side as the inturned tip is approached, and this gives the lower part a truncate or slightly convex appearance (Pl. X., 7, 10, 11; Pl. XI., 24). In *B. coloradensis*, instead of the lower part of the tips being flattened, there is developed a prominent ridge and this, together with a less prominent ridge on the upper side, gives the tip a very blunt appearance when viewed from the front (Pl. X., 8, 12; Pl. XI., 23). The tip in *B. coloradensis* is flattened on the anterior and posterior sides, while the tip in *B. Lindahli* is flattened dorsoventrally.

In *B. paludosa* the second division of the clasper tapers rather gradually to the blunt end, which is not inturned as in the other two species under consideration. The ratio between the length of the second joint and the first is for *B. coloradensis*, 1 to 1.68; for *B. Lindahli* 1 to 1.36; and for *B. paludosa* 1 to 1.28.

In young specimens of *B. coloradensis* the tubercle of the basal joint is often absent. It is also not uncommon to find such specimens with the second division ending rather bluntly, and not inturned. When in this condition, the second division resembles the corresponding division of *B. paludosa*.

The eggs of *B. paludosa* and *B. Lindahli* are of the same size (Packard, 1883: 340). Those of *B. coloradensis* are much larger, the ratio being 1.74 to 1.

The average length of the ovisac of *B. coloradensis* was 6.17 mm., varying from 4 mm. to 8 mm.; that of *B. Lindahli* was 3.71 mm., varying from 2 mm. to 5 mm., the ratio between the two species being 1.66 to 1. The ovisac of *B. Lindahli* is thicker in proportion to its length, and often contains 50 or more eggs arranged in from three to five rows, while the ovisac of *B. coloradensis* seldom contains as many as 30 eggs, and these generally arranged in from one to three rows (Pl. XI., 17, 18).

The caudal appendages of *B. Lindahli* are much longer in proportion to their breadth than those of *B. coloradensis* (Pl. XI., 19, 20, 21, 22). They are practically identical with the illustration of the caudal appendages of *B. paludosa* as given by Sars (1896 Pl. VI., 10).

Packard's original description of *B. Lindahli* (1883 : 339) is the only one known to the writer. For this description Packard had only the collection made at Wallace, Kansas, by Professor Lindahl. This collection contained 10 females and only 1 male. To judge from the description this male was an exceptionally small one and evidently not a typical specimen. It therefore seems best to give the more important specific characters of the male of this species :

Body long and slender, from 8 mm. to 17.5 mm. in length, less robust than that of *B. coloradensis ;* second antennæ long and powerful, reaching to the base of the fifth or sixth foot, 2.2 mm. to 5 mm. in length, somewhat shorter than those of *B. coloradensis ;* first division 1.2 mm. to 2.9 mm. in length, thick and provided on the outer margin with delicate sensory hairs, an elevated toothed area near the base on the inner side ; shorter than the first division of *B. coloradensis*, of about the same thickness but lacking the prominent tubercle at the base ; second division curved, shorter than the first, ratio to the first 1 to 1.36, the outer surface flattened and meeting the arched inner surface in two prominent angles. When viewed from the front or back, less than half as thick as the basal division. The flattened outer surface bends forward to the prominently inturned tip, which is more flattened below than above. This dorso-ventral flattening of the inturned portion causes it to appear very blunt when viewed from above or below, but to appear rather gradually pointed when viewed from the front or back. Eyes rather large, 0.325 mm. to 0.537 mm. in diameter, the ocular globe but slightly better developed anteriorly than posteriorly, and not much larger than the peduncle : much smaller than the eye of *B. coloradensis* — ratio 1 to 1.67 ; caudal appendages narrow-lanceolate, length plus setæ 1.62 mm. to 2.6 mm.; length of appendage 0.975 mm. to 1.62 mm.; longer in proportion to the breadth than those of *B. coloradensis.*

This species can be distinguished easily from *B. coloradensis* by the smaller eye and egg, by the difference in the shape of the eye, and by the greater number of eggs in the ovisac; by the absence of the basal tubercle on the second attennæ of the male, by the difference in the tips of the second attennæ, and by the longer and more slender caudal appendages.

It can be distinguished from *B. paludosa* by the absence of the teeth on the inner margin of the basal division, and by the inturned distal end of the second division of the second antenna.

Of the 82 females of *B. coloradensis* only two had both caudal appendages perfect, and only 6 of the 164 single caudal appendages were perfect. Forty males of *B. coloradensis* had only 11 imperfect appendages. No imperfect appendages were found on *B. Lindahli.*

A careful examination of the larvæ of *B. coloradensis* showed only perfect caudal appendages. From this it seems practically certain that the appendages of the adults were originally perfect. The tips of the appendages were first to disappear, and the appendages were then gradually shortened until only a small portion, or nothing at all was left (Pl. XII., 25 to 33). In all but a comparatively few, this change seemed to be a normal process. There was no sign of outward injury, but all the appearance of a gradual removal or absorption of the living substance. This appeared to be the same process by which the large swimming second antennæ of the larvæ are reduced to the much smaller and more simple second antennæ of the adult female.

A number of the appendages of both males and females showed parts of the appendage blackened by disease (Pl. XII., 29, 34 to 38), but this could not be mistaken for the other change. This blackening appeared in only a comparatively few of the appendages, and the number of males and females thus affected was about equal.

The caudal appendages of *B. coloradensis* first make their appearance as little knobs at the end of the common unsegmented body mass (Pl. X., 1, 2). The point of the knob is pushed out to form a stout, short, curved projection which will ultimately develop into the end or one of the end bristles of the mature appendage. By the time twelve body segments have developed, another small point is formed outside of the first bristle (Pl. X., 3). When all the abdominal segments can be seen, two more points have developed, one on either side (Pl. X., 4). These bristles now develop alternately until all are formed; the constriction between the appendage and the abdomen does not appear until the appendage is almost mature (Pl. X., 5, 6).

B. coloradensis was collected in Dead Lake, a small body of water in the Pike's Peak region. This lake has already been described in some detail by Ward (1904: 131, 135, Pl. XXIX., XXIV., XXV). Without visible outlet or inlet, it lies on the divide between Ruxton Creek and Beaver Creek at an altitude of about 3,350 m. (11,000 feet U. S. Topographic Map). On the southeast side is the ridge leading to the summit of Bald Mountain. This ridge is covered with a dense coniferous forest, shading the lake during the morning. The lake measures about 100 m. by 75 m. and does not exceed 2 m. in depth. The forest barely touches the southeast shore. The remainder of the shore is a mountain meadow, with a few shrubs here and there, and numerous large boulders lying in and about the lake. The bottom of the lake is of blue clay, probably derived from the decomposition of the granite, which is the only rock of this region. It has the appearance of an old lake, one about to disappear (Ward, 1904: 132). There is very little change of level, and the lake is not known to have been dry. For the last 14 years during which time the writer has repeatedly visited this place, conditions have not changed in any marked degree. During the summer of 1904 a sawmill and air pump were operated on the shore, without affecting the life in any marked degree. The water is clear, pure and very slightly alkaline. On July 12, 1904, one drop of dilute H_2SO_4 in which 1 cc. = 50.954 mg., was sufficient to neutralize 100 cc. of lake water, with methyl orange used as an indicator.

The earliest collections were made on May 20, 1904, the day when the ice broke, and while the southeast side was still partially covered with ice. The temperature of the water was 4°.7 C. in the warmest places, and varied to almost 0° C. in the coldest. Many larvæ had appeared, among which were those of *B. coloradensis*. These varied in stages of development from those in which only the antennæ, mandibular legs, and unsegmented body could be seen, to those in which all the segments had been formed.

On June, 4, the ice had all disappeared except at the southeast edge, and the warmest temperature was 6°.2 C. In this collection the larvæ were well developed, but none of those taken had transformed the swimming antennæ. They were larvæ of the last stage (Sars, 1896 : 55, Pl. VI., 5, 6). At the time of the next collection, June 17, the water temperature was 12°.2 C. and none of these forms were taken in the Birge net. While the mature animal was never captured in this way the larvæ of the last stage were easily taken in the net, so that, these forms were probably mature at this time.

The mature animals were extremely abundant July 12, with a surface temperature of 13°.6 C., and were equally abundant July 29, when the temperature was the same. At this time there was only 0°.2 C. difference between the surface and bottom temperatures. On August 12, with a surface temperature of 15.°6 C. the *Branchinecta* had disappeared. After a diligent search, one was seen, but although the search was continued no others were found. The bottom collections made at this time were filled with decomposing fragments of *Branchinecta.*

Ward (1904 : 139) recorded this form for July 13, 1903, when the surface temperature was 14°.4 C., and they were collected by the writer on August 13 of the same year.

From the above, it is clear that segmentation and most of the embryonic development must have taken place under the ice. the writer has no records of the temperature of the water before the ice broke, but this temperature according to Zchokke (1900 : 45) probably did not exceed 2°.2 C. By the time the temperature had risen to 6°.2 C. the larvæ were in the last stage of their development, and at 13°.6 C. the animals had become fully mature. They had disappeared when the temperature had risen to 15°.6 C. This was the warmest temperature recorded for this Lake. The mature forms were never abundant in the open water. They collected about the rocks and near the shore where they could be seen in great numbers swimming on their backs.

On August 12, 1903, as well as on July 12 and 29, 1904, the setæ of the legs and caudal appendages bore numbers of a green alga *Characium ambiguum* Hern. On July 29, these algæ were so numerous that many of the *Branchinecta* could swim only with slow labored movements, and this may have contributed to the sudden disappearance of the phyllopods.

This lake has no vertebrate fauna of any kind. The most common species besides the one here considered is *Diaptomus shoshone* Forbes (Ward, 1904 : 140) which is a very large form, of a pure deep red color, and very abundant. *Daphnia pulex* De Geer (Ward, 1904 : 149) and *Daphnia longispina* Müller are also quite abundant. The bottom fauna is especially rich in insect larvæ and Turbellaria. The flora is entirely algal, chiefly Conjugatæ, which are relatively abundant. The Schizophyceæ are well represented ; *Merismopedia glauca* Naeg., *Gomphosphæra aponica* Kuetz., *Anacystis pulverens* (Wood) Wolle, and a number of *Oscillatoria* and other filamentous forms were recorded.

The writer wishes to acknowledge his indebtedness to Dr. H. B. Ward, who suggested a study of the lakes in the Pike's Peak region and the comparison of *B. coloradensis* with *B. Lindahli*, who furnished the material of *B. Lindahli* collected by Professor Nelson, and who has given invaluable assistance in the preparation of these notes.

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EXPLANATION OF THE PLATES.

The figures are original drawings from material preserved in formalin. The outlines and some details were made with the assistance of the Abbé camera lucida, and the drawings were completed at the time from the same specimens.

PLATE X.

FIG. 1. Posterior end of the body of a larva of *C. coloradensis* showing the developing caudal appendages. × 119. At this stage the body mass was unsegmented and the first and second antennæ and mandibular legs alone could be distinguished.

FIGS. 2–6. Developing caudal appendages of *B. coloradensis.*

FIG. 2. At the time when six body segments can be seen. × 119.

FIG. 3. At the time when twelve body segments can be seen. × 119.

FIG. 4. At the time when all abdominal segments can be distinguished. × 119.

FIG. 5. Later stage. × 35.

FIG. 6. A still later stage. × 35.

FIG. 7. Male second antenna or clasper of *B. Lindahli; ta,* toothed area. × 20.

FIG. 8. Male second antenna or clasper of *B. coloradensis; t,* tubercle; *ta,* toothed area. × 20.

FIG. 9. Distal end of the male second antenna of *B. Lindahli* viewed from the front. × 35.

FIG. 10. The same viewed from the outside. × 35.

FIG. 11. The same viewed from the front and below. × 35.

FIG. 12. Distal end of the male second antenna of *B. coloradensis* viewed from the outside. × 35.

FIG. 13. Eye of the female of *B. Lindahli*, dorsal view; *a*, anterior side; *p*, posterior side. × 35.

FIG. 14. Eye of the male of *B. Lindahli*, dorsal view; *a*, anterior side; *p*, posterior side. × 35.

FIG. 15. Eye of the female of *B. coloradensis*, dorsal view; *a*, anterior side; *p*, posterior side. × 35.

FIG. 16. Eye of the male of *B. coloradensis*, dorsal view; *a*, anterior side; *p*, posterior side. × 35.

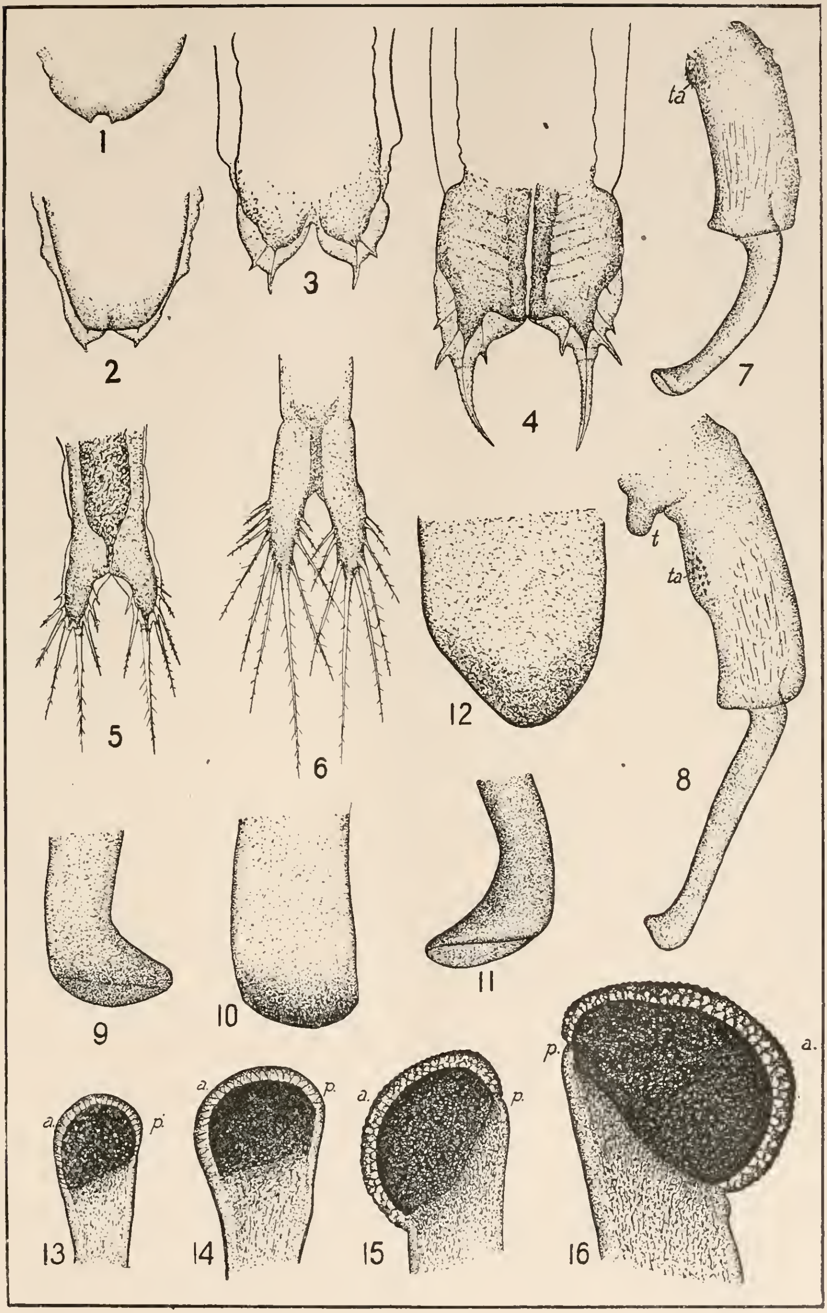
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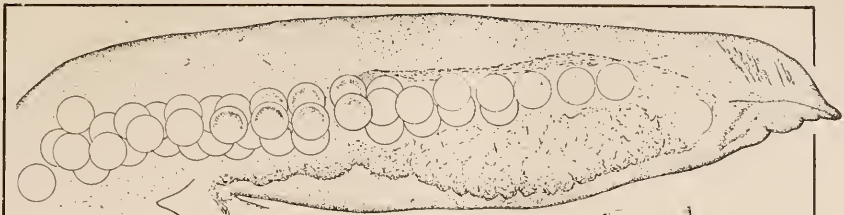
Plate XI.

Fig. 17. Ovisac of *B. Lindahli* with eggs. × 24.
Fig. 18. Ovisac of *B. coloradensis* with eggs. × 24.
Fig. 19. Caudal appendages of male of *B. coloradensis*. × 21.
Fig. 20. Caudal appendages of female of *B. coloradensis*. × 21.
Fig. 21. Caudal appendages of male of *B. Lindahli*. × 21.
Fig 22. Caudal appendages of female of *B. Lindahli*. × 21.
Fig. 23. Head of male of *B. coloradensis*, front view. × 14.
Fig. 24. Head of male of *B. Lindahli*, front view. × 14.



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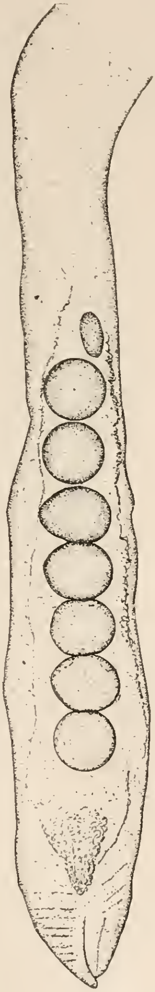

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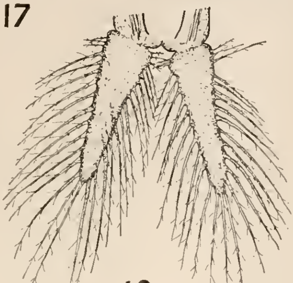

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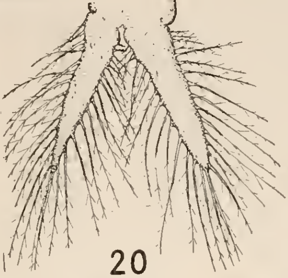

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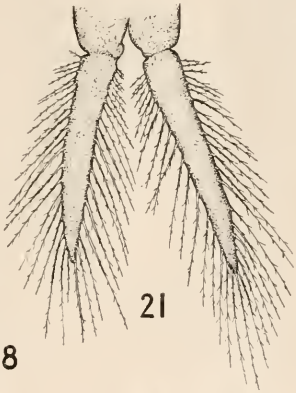

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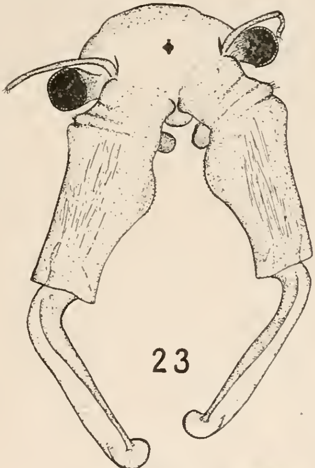

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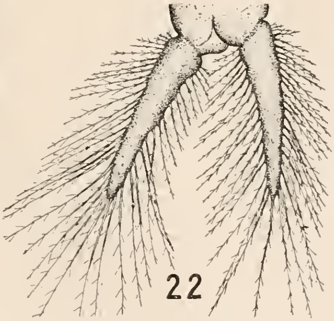

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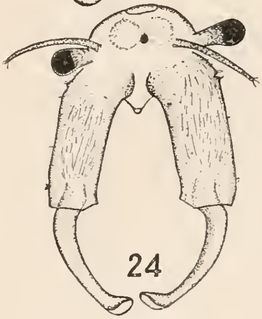

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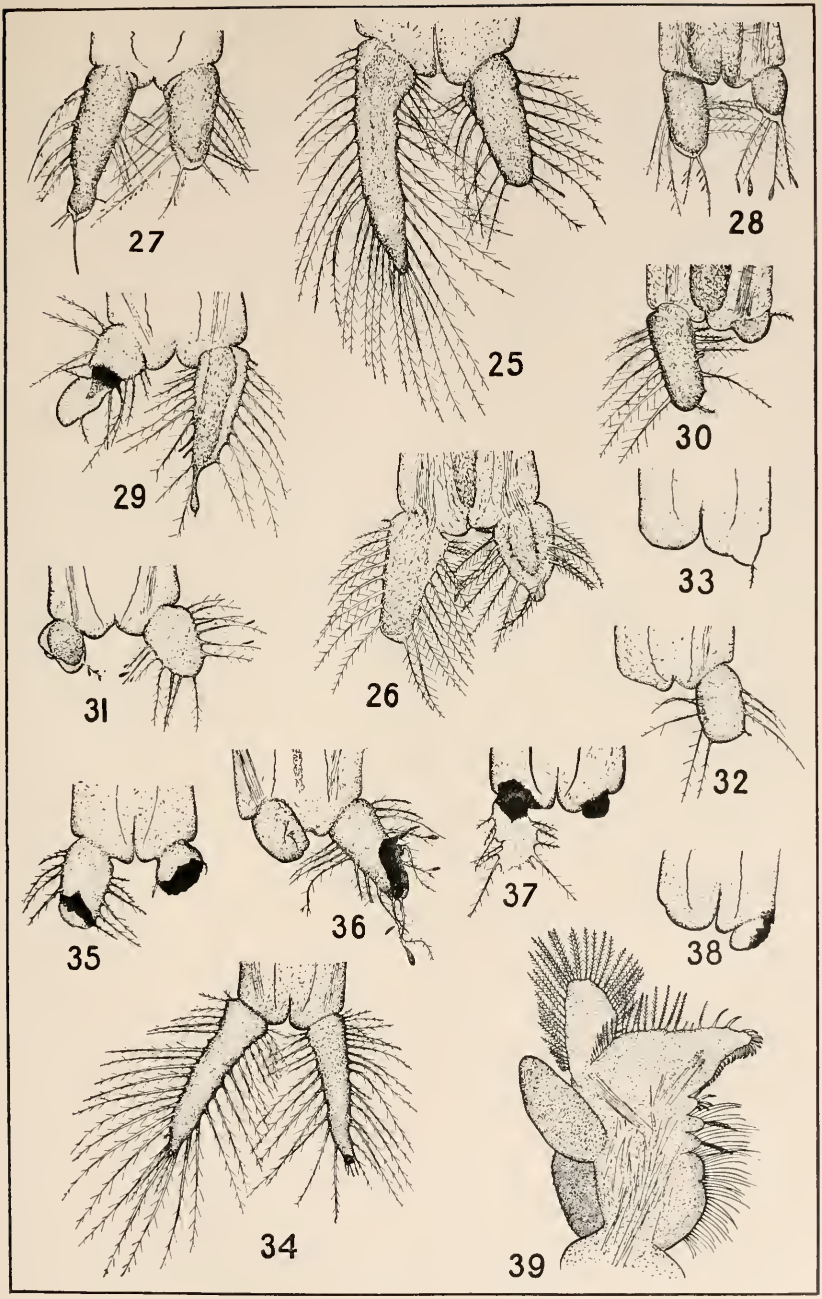
PLATE XII.

FIGS. 25–28, 30–33. Caudal appendages of females of *B. coloradensis* in various stages of absorption. × 25.

FIGS. 29, 34–38. Diseased caudal appendages of females of *B. coloradensis.* × 25.

FIG. 39. Branchial leg of the sixth pair of the male of *B. Lindahli.* × 14.



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BIOLOGICAL BULLETIN

STUDIES IN THE INHERITANCE OF COLOR IN PERCHERON HORSES.

E. H. HARPER.

I. THE INFLUENCE OF SELECTION AND INBREEDING UPON PREPOTENCY.

The materials for this paper have been drawn from the records of the American and French Percheron Horse Breeding Associations. The data obtainable from breeders' books have been used extensively within recent years in the investigation of problems of heredity. The "Law of Ancestral Heredity" formulated by Galton and revised by Karl Pearson is the foremost of the deductions claimed as a result of the study of such data.

The information furnished by the Percheron register is in respect to color, sex and age, and the questions proposed in this paper involve the three sorts of data available. Three questions have been made the subject of investigation: (1) To what extent a color which has been predominant in the breed in the past shows any prepotency over a color which has been increased by breeders' selection. The case with the Percherons is that gray has been the predominant color, but black is on the increase. (2) A second question has been raised as to normal inheritance from parents of different ages, whether parents of greater age influence the offspring to a greater extent, and whether there is an optimum age of prepotency. (3) Whether either sex is prepotent over the other.

At the outset of this investigation the writer started to test the "Law of Ancestral Heredity" of Galton. To this end was collected the complete ancestry of 179 individuals up to the third ancestral generation, showing the colors of parents, grandparents and great-grandparents. The results showed that a secular change has been in progress in the breed increasing the per cent. of black individuals. The figures are given in Table I.

The per cent. of grays among the great-grandparents was 58, and this declined to 47 per cent. among the grandparents and to 39 per cent. among the parents. Corresponding to these there was a rise in the per cent. of blacks from 37 per cent. among the great-grandparents to 46 per cent. of the grandparents and 49 per cent. of the parents. There was also an increase in the bay-brown group from 6 per cent. to 12 per cent.

Table I.

		G.-G.-P.	G.-P.	P.	Offspring.
White or Gray.	No. of Sires.	368	140	68	♂ 34
	No. of Dams.	458	200	73	♀ 30
	Total.	826	340	141	64
	Per Cent.	57.68	47.48	39.38	35.75
Black.	No. of Sires.	327	190	81	♂ 40
	No. of Dams.	198	140	93	♀ 51
	Total.	525	330	174	91
	Per Cent.	36.66	46.08	48.60	50.83
Bay, Brown and Chestnut.	No. of Sires.	21	28	30	♂ 9
	No. of Dams.	60	18	13	♀ 15
	Total.	81	46	43	24
	Per Cent.	5.65	6.42	12.01	13.40
	Totals.	1,432	716	358	179

These facts correspond to the testimony of breeders in regard to the increasing popularity of black. The preference or fashion for black seems to be leading to a gradual change from the formerly predominant gray color of the breed which prevailed in its place of origin in France. The gray-white group of colors includes a number of minor varieties and is the outcome of former generations of inbreeding of animals of this color.

The 179 offspring whose ancestry has been traced are themselves divided as follows among the three color groups: Gray 64, black 91, bay-brown 24. This is a showing of 51 per cent. of blacks among the offspring, which is in excess of the per cent. found among the parents. Doubtless this is an exhibition of the breeders' preference for black which has led to an excessive registration of animals of the favorite color. The expense of registration must be considered as a factor. Thus the 179 individuals are not in all probability a representative population, owing to the elimination of grays from the register.

It should be added that this is the surface explanation of the facts, which might be accounted for partly at least in other ways, as by an actual prepotency of the black color, or by a melanic tendency due to effect of change of climate on the breed on removal from its original home in France to America. All of the offspring and parents and almost all of the grandparents are American registered animals. It is chiefly in the great-grandparental generation that the French records have been drawn upon.

The following description of the Percheron breed is taken from a work on "The Horse" by Roberts (Macmillan, 1905): "About 1820 two noted gray Oriental stallions, Godolphin and Gallipoli, were introduced into the Government stables at Pin. These two prepotent stallions fixed the style of color and fastened it on an already susceptible breed (p. 159). . . . The color of most Percherons is gray of varied shades. Sometimes it is quite light, becoming nearly pure white in old age. Again, the striking light and dark dapples are seen, and dark grays, almost black, with a few white hairs. Comparatively few blacks have, as yet, been bred, although dark colors are sought and are more common than formerly. The American purchaser prefers darker rather than lighter colors; hence the effort in France is to produce darker colored animals than formerly. . . . It will take many generations to entirely eliminate the light colors, so long one of the characteristics of the breed; but this will be accomplished in time if Americans persist in preferring dark- rather than light-colored draft-horses. This preference is not founded on a fad, for, other things being equal, dark-colored horses are to be preferred to light-colored ones" (p. 162 ff.).

From all that we know of the history of the breed it is quite obvious that the increase in black and decrease in gray through four generations, shown in Table I., is at least partly a result of breeders' selection, and would not warrant the assumption of a melanic tendency or prepotency of black. Indeed, it is quite equally obvious that we should expect the long predominant color, gray, to be the prepotent color. Independent data have been collected which bear upon this point, and the same data have been tabulated with reference to the two other matters mentioned at the outset, the prepotency of sex and the relation of age to prepotency.

THE SELECTION OF DATA TO DETERMINE PREPOTENCY.

With the view of determining certain of the factors of prepotency, two thousand individuals of pure black or gray color have been selected at random, consisting of one thousand colts and one thousand fillies, the parents likewise being of pure color, one black the other gray. This method of selection gives a positive resemblance of the offspring to the one or the other parent. It seemed to the writer that the pure colors were the only ones from which inferences could be drawn, since the descriptions of color are too meager to warrant a safe inference in cases of parents or offspring of mixed color. It would be impossible in the case of mixed colors to decide which was the preponderating color or to infer the resemblance of the offspring to one or the other parent. This defect is apparently inherent in data not originally collected for any scientific purpose. Any consideration of the Mendelian hypothesis is precluded. And while that fascinating theory would naturally be the first to turn one's attention to investigations of this sort, it has seemed that the limited use of data as above outlined might prove to be warrantable as a method for certain purposes proposed in this this paper. All of the data presented in this paper outside of Table I. consist of that just referred to, namely, the 2,000 individuals selected at random according to the method described, and including 1,000 colts and 1,000 fillies.

TABLE II.

PREPOTENCY IN RELATION TO SEX.

	Sire Prepotent.	Dam Prepotent.	Totals.
Colts	457	543	1,000
Fillies	463	537	1,000
Totals	920	1,080	2,000
Per Cent.	46	54	

In Table II. the data are arranged with reference to sex prepotency. It is seen that of 1,000 colts 543 or 54.3 per cent.

resemble the dam, and of 1,000 fillies 537 or 53.7 per cent. resemble the dam, or the dam is prepotent in 54 per cent. of all cases. The ratio between the prepotency of dam and sire is a little less than 5:4.

In the next table the sexes are separated on the basis of color and we find that gray dams and black sires are greatly in excess, being nearly 75 per cent. of the whole number.

TABLE III.—*Colts.*

PREPOTENCY IN RELATION TO BOTH SEX AND COLOR.

Sires.	Black.	Per Cent.	Gray.	Per Cent.	Totals.
Prepotent..........	333	44.04	124	50.81	457
Non-prepotent ...	423		120		543
Totals.	756		244		1,000
Dams.					
Prepotent.	120	49.19	423	55.96	543
Non-prepotent ...	124		333		457
Totals.	244		756		1,000

TABLE IV.—*Fillies.*

Sires.	Black.	Per Cent.	Gray.	Per Cent.	Totals.
Prepotent..........	322	44.98	141	49.65	463
Non-prepotent	394		143		537
Totals.	716		284		1,000
Dams.					
Prepotent.	143	50.35	394	55.02	537
Non-prepotent	141		322		463
Totals.	284		716		1,000

Taking the table for the 1,000 colts first, we find that there are 756 black sires and 244 gray sires, and correspondingly 756 gray dams and 244 black dams. The gray dams moreover are prepotent in 56 per cent. of the cases and the black dams in only 49 per cent. The gray sires are prepotent in 51 per cent. of the cases and black sires in 44 per cent. These figures do not essentially alter the previous results as to the prepotency of the dam but indicate that the gray dam is more prepotent than the black dam and the gray sire than the black sire. But the gray sire is less prepotent than the gray dam (51:56). The relations may be expressed thus: The combination of prepotent sex + subpotent color (B ♀) = subpotent sex + prepotent color (G ♂). For the prepotencies are nearly equal in this case (49.2:50.8).

The figures for the 1,000 fillies are nearly the same as for the colts (Table IV.). The black sires and gray dams are in excess as before, there being 716 black sires to 284 grays and correspondingly 716 gray dams to 284 blacks. The gray dams are prepotent in 55 per cent. of the cases, black dams in about 50 per cent.; gray sires in about 50 per cent., black sires 45 per cent. Here also the gray dams are more prepotent than the gray sires (55:50). Also the relation holds as before expressed. B ♀ = G ♂ (50.3:49.7).

Table V.

Prepotency in Relation to Color.

	Prepotent.	Per Cent.	Non-prepotent.	Per Cent.	Totals.
Gray parents	1,082	54.1	918	45.9	2,000
Black parents	918	45.9	1,082	54.1	2,000
Totals	2,000		2,000		4,000

In Table V. are condensed the data of III.-IV. arranged with reference to color alone. From this it is seen that gray parents are prepotent in 54.1 per cent. of the cases. Oddly enough this almost coincides with the prepotency of dams (Table II.) which was 54 per cent. This curious result comes from the fact pointed out above that black dams mated with gray sires are almost equal in prepotency in the case of both colts and fillies. Consequently the whole difference between the prepotency of gray and black parents arises from the unions between gray dams and black sires in which the gray dams are prepotent in 55–56 per cent. of the cases.

These results show from data independent of Table I. that black is not a prepotent color. Therefore the secular change shown in the increase of black in Table I. must be due to breeders' selection. It is also evident that breeders' preference is exercised by the more frequent use of black stallions. Although stallions are chosen for their individual superiority and prepotency it would seem that their superiority is not sufficient to make them prepotent as to color in the majority of cases. The original color of the race, gray, is prepotent, having behind it the hereditary force of previous generations of inbreeding. In the inheritance of color the tendency is to return to the original gray.

About 75 per cent. of the parents of grays are gray while only 60 per cent. of the parents of black individuals are black. This was found to be the case with the parents of 300 grays and 300 blacks selected at random from data not included in the rest of this paper. Thus the reason for the prepotency of gray is manifest from the average character of its ancestry.

The prepotency of the dam seems to be partly explained by the fact that gray dams are most numerous, but this does not wholly explain the dam's prepotency. For gray dams are prepotent in a higher degree than are gray sires (56:51 for colts; 55:50 for fillies).

It must be remembered that we are dealing with the recorded colors as yearlings and that a certain per cent. of color changes must occur later on. It would be interesting, for example, to have data bearing upon the question whether the young of both sexes tend to resemble the dam.

Pearson ('01) finds in respect to the inheritance of eye-color in man, "That the younger generation takes as a whole more after its male than its female ascendents and collaterals." In this paper the prepotency of the dam has been shown to be partly the result of association with a prepotent color, but this factor does not wholly explain the dam's prepotency although it diminishes her apparent prepotency.

The conclusion that the prepotency of the gray color is the effect of inbreeding owing to its long established existence as a racial characteristic in this breed may suggest the question as to how long would be required for the black color to become fixed by long continued selection and inbreeding. There may be some evidence as to the point in the data contained in Table XV. (see Appendix). Of the 91 black offspring 38 had both parents and at least half of the grandparents and great grandparents black. Moreover all the individuals having that amount of black in their ancestry were black. Of course this apparent result must be largely caused by breeders' selection, eliminating grays from the records. If it has any force at all as an exhibition of the effects of the cumulation of black in the near ancestry it would go to show that the selected color tends to become stamped in "indelibly" so to speak after a few generations of selective inbreeding. Any such facts, if proved, would of course militate against Galton's or Pearson's law which ascribes a continued influence to the remote ancestry. The influence of the ancestral generations diminishes according to Galton's hypothesis in the series — .50, .25, .125, .0625, etc. According to Pearson's, a greater influence is ascribed to the remote generations, the series (numbered II.) running as follows — .50 (parental influence), .33 (grandparental influence), .22 (great-grandparental influence), .15 (great-great-grandparental influence).

II. Is There a Correlation Between Age and Prepotency?

The remaining question proposed in this paper is whether there exists any relation between age of parents and prepotency. Whether there is an optimum age of prepotency in either parent and the power of influencing the character of the offspring increases up to this point.

The data have been first arranged in two groups for the two sexes. The 1,000 colts are treated in Table VI. (*a*) and the 1,000 fillies in Table VI. (*b*).

Table VI. (*a*).

Colts.

	Sire of Same Age, or Older.	Per Cent. of Prepotency.	Sire Younger.	Per Cent. of Prepotency.	Totals.
Sire prepotent.........	265	46.50	192	44.66	457
Dam prepotent........	305	53.50	238	55.34	543
Totals...............	570		430		1,000

Table VI. (*b*).

Fillies.

	Sire of Same Age, or Older.	Per Cent. of Prepotency.	Sire Younger.	Per Cent. of Prepotency.	Totals
Sire prepotent.........	283	47.89	180	44.01	463
Dam prepotent	308	52.11	229	55.99	537
Totals.	591		409		1,000

Table VI. (*a*) shows that dams are slightly more prepotent when older, or in 55.3 per cent. of the cases against 53.5 per cent. where the sire was older or of the same age.

In the table of the fillies VI. (*b*) the dam is seen to be prepotent in 56 per cent. of the cases when older, as against 52 per cent. when younger or of the same age with the sire. These differences are slight and consistent in case of both colts and fillies in making the dam more prepotent when older and correspondingly the sire less subpotent when older.

In order to see whether there appears to be an optimum age of prepotency the data have been arranged in a number of tables that follow. In the mating of domestic animals a greater variety of crosses with regard to the respective ages of the parents is of course met with than in man. In marriage the man is ordinarily older, but among horses we have all possible combinations occurring frequently.

In arranging the data to determine if there be an optimum age the parents have been grouped as follows: I., very young, 3–4 years old. II., young, 5–7. III., medium age, 8–10. IV., older, 11–13. V., very old, 14 and over.

In Table VII. which follows are given all cases in which dams were mated with very young sires.

Table VII.

Sires 3 to 4 Years.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts	77	106	57.92	183
Fillies	72	110	60.43	182
Totals	149	216	59.17	365

When the sire is very young 58 per cent. of the colts and 60 per cent. of the fillies resemble the dam, or the prepotency of the dam is 59 per cent. as against the average prepotency of 54 per cent. derived from Table II.

Naturally we would take next for consideration the prepotency of dams mated with very old sires, 14 and over. There were only 70 such cases in the 2,000. They show that 66 per cent. of the colts and 63 per cent. of the fillies resemble the dam, an average prepotency of the dam of 64 per cent.

Table VIII.

Sires of 14 Years and Older.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts	10	19	65.55	29
Fillies	15	26	63.41	41
Totals	25	45	64.28	70

Now leaving out very young and very old sires, the cases in which dams were mated with sires 5–13 years of age are given, first for colts and then for fillies.

TABLE IX.

COLTS. SIRES 5–13 YEARS OLD.

Age of Dam.	3–4 Years.	5–7 Years.	8–10 Years.	11–13 Years.	14 Years +.	Totals.
Sire prepotent.......	82	150	85	34	19	370
Dam prepotent......	80	159	104	55	20	418
Prepotency of dam.	49.38%	51.45%	55.02%	61.79%	51.28%	788

When the dams are grouped as above from young to old — the prepotency of the dam runs along in a series above shown — 49, 51, 55, 62, 51, apparently showing an optimum period of prepotency somewhere in middle age. In Table X. dams mated with sires of 8–10, a more homogeneous group, are considered.

TABLE X.

COLTS. SIRES 8–10 YEARS OLD.

Age of Dam.	3–4 Years.	5–7 Years.	8–10 Years.	11–13 Years.	14 Years +.	Totals.
Sire prepotent.......	24	51	20	13	8	116
Dam prepotent......	15	58	38	22	7	140
Prepotency of dam.	38.46%	53.21%	65.51%	62.85%	46.66%	256

Here the dam's prepotency makes a series — 38, 53, 66, 63, 47, the maximum being in middle age. In the next table the results for fillies are given.

TABLE XI.

FILLIES. SIRES 5–13 YEARS OLD.

Age of Dam.	3–4 Years.	5–7 Years.	8–10 Years.	11–13 Years.	14 Years +.	Totals.
Sire prepotent.......	80	153	95	39	9	376
Dam prepotent.. ..	88	142	114	40	21	401
Prepotency of dam.	52.4%	48.1%	54.54%	50.6%	70%	
	(average) 49.67			(average) 55.96		777

It may be noted that in the last table the prepotency of the dam does not show a regular increase nor run to so high a point at any age as in the table of colts. On the whole, however, there is a rise in the prepotency of the dam with age.

In the previous tables dams were taken of all ages. In the next are given all the cases where very young dams were mated with very young sires.

TABLE XII.

Sire and Dam 3 to 4 Years.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts	20	33	62.26	53
Fillies	22	31	58.49	53
Totals	42	64	60.37	106

The dam's prepotency is high, where the ages are on an equality and just as high as in Table VII., where the dam had the supposed advantage of age.

Next is given a table showing very young dams mated with sires 5–13, the more prepotent sires.

TABLE XIII.

Dam 3 to 4, Sire 5 to 13 Years.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts	82	80	49.38	162
Fillies	80	88	52.32	168
Totals	162	168	50.90	330

This table shows an equality as to prepotency between very young dams and the more prepotent sires. In Table XIV. very young dams mated with sires of all ages are included.

TABLE XIV.

Dam 3 to 4 Years.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts	103	119	53.60	222
Fillies	105	124	54.10	229
Totals	208	243	53.87	451

Now let us compare the results of the last three tables, XII.–XIV., and see if there is any consistent relation between these results. Arranging them so as to show an increasing prepotency for the dam, we find that dams 3–4 years of age mated with the more prepotent sires 5–13 (Table XIII.) are least prepotent. Next comes the case of young dams mated with sires of all ages, with a prepotency of 53.8 per cent. Still more prepotent are young dams mated with their equals in age (60 per cent.)

The number of cases in which very old dams occur is too small to permit a tabulation in this manner. The results for these and for dams of medium age can be seen by inspection of Tables IX.–XI. without any farther tabulation of the data.

We find the following idea expressed by Redfield ('03) in respect to the influence of age upon prepotency: "As between two individuals of the same breed, the same rule probably holds, that the individual which has had its characteristics more firmly fixed by inbreeding will be prepotent. In the life of an individual a character is more firmly fixed in comparative old age than in youth. Consequently we may assume in the absence of evidence to the contrary, that other things being equal, the older individual will be prepotent over the younger one." Redfield's work deals with human statistics and he aims to prove use-inheritance in the case of acquired mental powers.

It is apparent of course that color is not an acquired character except to a limited extent in regard to which the present data show nothing. It is rather obscure as to what may be meant by a character becoming more fixed by age. But that the power of a parent to influence the character of the offspring may be correlated with the time of life and consequent vigor is a matter seemingly independent of whether acquired or congenital characters alone may be transmitted. On the whole these data show that the influence of age is confined within narrow limits at least.

Conclusions.

1. There is a secular change of color in progress in the breed resulting from breeders' preference for black.

2. Gray, the long-established color, is prepotent over black.

3. The dam is prepotent over the sire in the ratio of about five to four. Gray dams are more prepotent than black dams and gray sires than black sires; also gray dams are more prepotent than gray sires and black dams than black sires. The dam's prepotency is partly due to association with the long predominant color. Gray dams and black sires are greatly in excess (nearly 75 per cent. of all).

4. There is apparently a degree of correlation between age and prepotency.

5. There appears to be an optimum age of prepotency, occurring in middle life.

In an appendix are added some further tabulations of the data of Table I., testing those results by Galton's Law.

The data given in Table I. were collected under the direction of Prof. C. B. Davenport at Chicago, with a view to testing the application of Galton's Law of Ancestral Heredity, but since the manifest action of breeders' selection has vitiated the data for deductions as to normal inheritance, for a series of generations, it is not worth while to include here any consideration of Galton's Law.

I wish to thank Mr. S. D. Thompson, Secretary of the Percheron Horse Breeders' Association, for access to unpublished records.

Appendix. The Data of Table I. in Relation to Galton's Law.

It was stated at the outset of this paper that the results of Table I. do not agree with Galton's Law of Ancestral Heredity, showing an abnormal proportion of black offspring, a fact doubtless due to breeders' selection. The excess of blacks is clearly seen by inspection without the use of Galton's method of calculation, since the per cent. of black offspring is greater than the per cent. of black parents, and the latter are greatly in excess of the number present in the two preceding generations. Galton's method of calculation was applied to these data, but it is not worth while to include those calculations here in view of the fact that the manifest action of breeders' selection has vitiated the data for deductions as to normal inheritance. The general result of the calculations may be given, however. They were made according to Galton's original method, not by Pearson's method of correlation, by which he makes use of far more data than the ancestry of 179 individuals would furnish.

The results are briefly: Out of 179 offspring the number of blacks calculated was 74, the actual number being 91. Of the grays the number calculated was 77, the actual number was 64. The bay-brown group exceeded calculations considerably, comprising the remaining 24 individuals.

At the right in Tables XV.–XVI. are given the per cents. of blacks and grays in the consecutive classes beginning with those having the highest amount of the same color in the ancestry and running down. The series run:

Grays,	83	65	61	44	17	2	0
Blacks,	100	91	79	38	( )	14	0

Comparing these series it is seen that the top class of blacks comprises 100 per cent. of black individuals and, throughout, the series runs higher than for the grays. This may be interpreted as the result of breeders' selection either wholly or in part, a

Table XV.

Showing Number of Blacks in Ancestry of 179 Individuals, for Three Ancestral Generations.

No of Black Parents.	No. of Black Grand-Parents.		No. of Black Great-Grandparents.										Per Cent. of Blacks.
			8	7	6	5	4	3	2	1	0	Totals	
2	4	*a*		1	1	1	6	4	1		1	15	100
		b		1	1	1	6	4	1		1	15	
	3	*a*				9	1	3		1		14	
		b				9	1	3		1		14	
	2	*a*				4	5	5	2	2		18	90.9
		b				4	5	4	1	2		16	
	1	*a*					1		1	1		3	
		b					1		1	1		3	
	0	*a*							1			1	
		b							1			1	
1	4	*a*							1			1	78.9
		b							1			1	
	3	*a*				4	8	5	1			18	
		b				3	6	4	1			14	
	2	*a*			1	7	5	3	6			22	38
		b			1	3	2	1	3			10	
	1	*a*				3	1	6	3	3	2	18	
		b				2	1	3	1	0	0	7	
	0	*a*					2		3	3	2	10	
		b					1		1	0	0	2	
0	4	*a*											
		b											
	3	*a*											
		b											
	2	*a*				2	2	2	7	7		20	13.5
		b				1	2	1	1	1		6	
	1	*a*				3	3	3	11	1	6	27	
		b				0	1	1	0	0	0	2	
	0	*a*						1	7		4	12	0
		b						0	0		0	0	
	Totals.	*a*										179	
		b										91	

Note.— *a* equals number in class; *b* equals number of blacks.

matter which is wholly beyond determination. It has been discussed above whether these results show the effects of inbreeding of blacks (p. 271).

Galton's law and Pearson's modification of it have both been criticised by advocates of the Mendelian hypothesis (Castle, '03). It is not included in the purpose of this paper to consider that law except in incidental connection with Tables I., XV. and XVI.

Table XVI.

Showing Number of Grays in Ancestry of 179 Individuals, for Three Ancestral Generations.

No. of Gray Parents. I.	No of Gray G. P. II.	No. of Gray Great-Grandparents. III.											Per Cent. of Grays.
			8	7	6	5	4	3	2	1	0	Totals.	
2	4	*a*	3		4	1						8	83.3
		b	3		3	1						7	
	3	*a*	5		6	3	1		1			16	
		b	4		6	2	0		1			13	
	2	*a*		2	8	1	5		1			17	64.7
		b		2	3	1	4		1			11	
	1	*a*											
		b											
1	4	*a*		2	4	1	1					8	60.7
		b		2	4	0	0					6	
	3	*a*	5		6	5	1	3				20	
		b	4		3	3	1	0				11	
	2	*a*			8	2	3	7	3			23	43.7
		b			5	2	1	4	1			13	
	1	*a*			1	2	4	1		1		9	
		b			0	0	1	0		0		1	
0	4	*a*			1							1	16.7
		b			0							0	
	3	*a*			3		1	1				5	
		b			1		0	0				1	
	2	*a*		2	2		10	4				18	2.3
		b		0	0		1	0				1	
	1	*a*			1	2	6	14	2			25	
		b			0	0	0	0	0			0	
	0	*a*		1	1	3	7	12	3	2		29	0
		b		0	0	0	0	0	0	0		0	
	Totals.	*a*										179	
		b										64	

Note.—*a* equals number in class; *b* equals number of grays.

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Evanston, Ill., June, 1905.

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SECTIONING PARAFFINE AT A TEMPERATURE OF 25° FAHRENHEIT.

KATHARINE FOOT AND E. C. STROBELL.

It is with some hesitation that we publish our troublesome device for cutting thin sections of the egg of *Allolobophora*, for in other material investigators are able to obtain thin sections without recourse to such complicated methods.

Our method is quite opposed to established rules, for the best authorities on technique recommend sectioning in a temperature varying, according to the season, from 60° to 70° Fahrenheit, in some cases employing heat reflected from a lamp, and they advise the use of paraffine having a melting point not higher than 45° to 50° C., emphasizing the fact that harder paraffine is "most hurtful to tissue."[1]

We found it necessary, however, to use paraffine of a much higher melting point, working slowly toward the use of this harder paraffine, and comparing the results step by step, in order to demonstrate whether the hard paraffine was really harmful to the egg structures. As no injury to the cytoplasmic or nuclear structures could be detected, we finally imbedded the eggs in the hardest paraffine obtainable, that having the melting point registered at 74° C. With this paraffine, a Thoma microtome, and the knife in the best possible condition, we were able to get moderately good sections of 5 and 6 μ, but an attempt to cut thinner sections crushed the paraffine enough to destroy the spherical form of the egg. What was gained by the thinner sections being more than sacrificed by the distortion of nearly all the constituents of the egg.

We tried to increase the hardness of the paraffine by devising an object carrier that would hold a piece of ice or iced water, but there was little gained by this method. The paraffine could be safely cooled only to a very limited degree below the temperature of the room, beyond this, moisture formed on the block and serial sections were impossible. We also tried Bütschli's method of painting each section with a thin layer of colloiden. In this way Bütschli obtained satisfactory sections less than 1 μ thick, but we failed to get like results.

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[1] Bolles Lee, "The Microtomist's Vade-Mecum." 1896.

We were convinced that the difficulties were not due to mechanical defects in the microtomes, for we were able to cut sections 3, 2 and even 1 μ with almost every microtome we have tried. But in these thin sections, the perfect contour of the eggs was destroyed, their diameter in some cases being reduced more than one half. This we demonstrated by cutting a series of sections, the first half dozen 10 μ, the next half dozen 5 μ, then 4, 3, 2 and 1 μ. Comparing the last sections of the series with the 10 μ section showed how much the structure of the egg was sacrificed to the thin sections, and convinced us of the necessity of devising some special method to harden the paraffine in order to secure thin sections in every way up to the standard of the 10 μ section.

Experimenting with a block of pure paraffine less than one eighth of an inch square (without any imbedded object) and setting the microtome at 3 μ, we gradually lowered the surrounding temperature until each section of the paraffine maintained the exact size of the original block.

The text-figure on page 283 illustrates the freezer we finally designed to enable us to cut serial sections of 1½ to 2½ μ at a temperature about 25° Fahrenheit. The work table, on which the freezer is operated, is placed close to a north window, and on the table we put a heavy cotton pad, covering this with a heavy rubber sheet. Then the microtome (Thoma in our case) is set in place on the rubber sheet. Near the microtome we arrange the wooden object carriers, each with its subject ready for final cutting; the necessary number of clean slides and boxes (with close covers) just large enough to hold a slide.[1] Then the freezer is put in place over the microtome, the work table forming the bottom of the operating compartment *D*. The ice chamber *B* is then packed with alternate layers of cracked ice and salt, the corks tied securely in the hand holes *H*, and a rubber tube fitted over the drain tube *M* with the free end in a pail under the

[1] We use for this purpose the shallow tin boxes formerly made for typewriter ribbons.

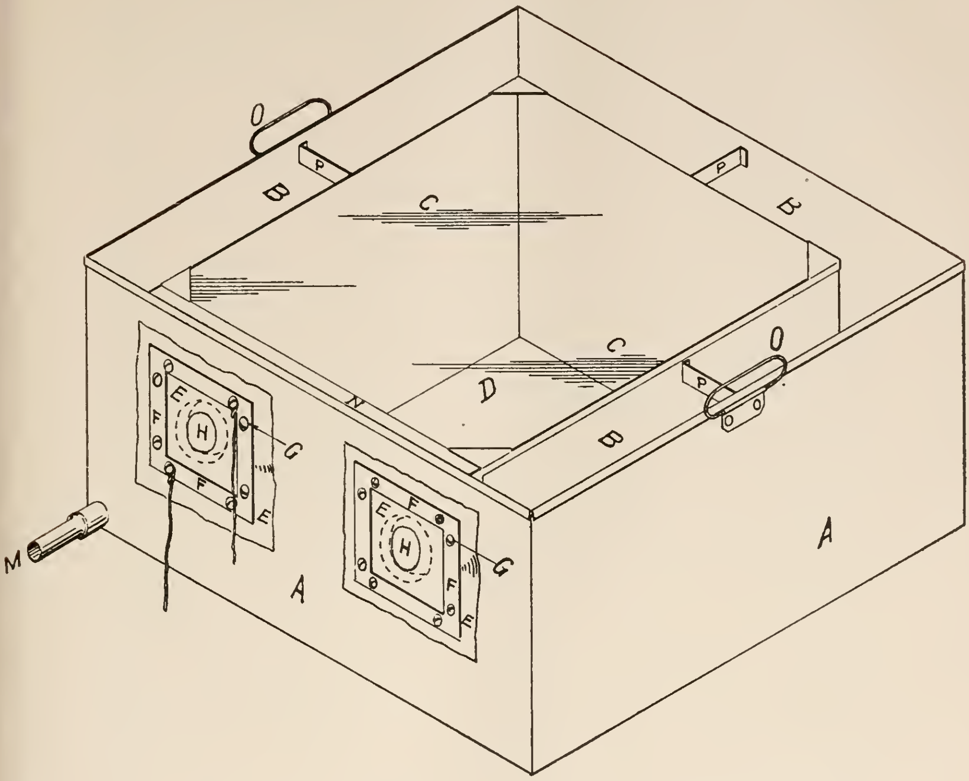

FIG. I. The above figure is drawn to scale one eighth full size. *A*, exterior of copper box. *B*, ice chamber. *C*, plate glass ¾ inch thick, forming the top of operating compartment *D*. *D*, operating compartment The work table on which the microtome is placed forms the bottom of the operating compartment, which has the same area as the plate glass top, *C*. A thermometer is suspended in the compartment just above the microtome. This is done by glueing two loops of tape to the plate glass cover *C* and slipping the thermometer through these loops. We sometimes place a second thermometer on the bottom of the operating compartment, but the temperature registered by the one near the knife is the proper guide. *E*, heavy sheet rubber, which is kept in place by the copper frame, *F*, and copper bolts, *G*. *F*, copper frame. *G*, copper bolts which pass through the frame *F*, sheet rubber, *E*, and air chamber, *N*. The free ends of these bolts with nuts are in the operating compartment, *D*. *H*, hand holes in the heavy sheet rubber. The outer broken circle indicates the larger holes through the copper air chamber, *N*, and these must be large enough to admit the forearm freely. The hand hole, *H*, in the sheet rubber, must, on the contrary, be small enough to clasp the wrist tightly, to prevent escape of cold air from the operating compartment *D*. The sheet rubber must be renewed at least once a year, or as often as it becomes hard and loses its elasticity. When not operating the microtome, the hand holes, *H*, must be tightly closed with

table. After this the freezer must be covered with a heavy quilted covering and allowed to stand undisturbed for several hours. As a rule we "set" the freezer at night, and the following morning sectioning can begin at once.

When sectioning it is necessary to wear heavy gloves covering not only the hands, but the wrists and forearm. This obviates the discomfort of cold hands and prevents a too rapid rise of temperature in the freezer from the warmth radiating from the hands. To prevent frost forming on the glass cover, we have found it advisable to select cold days for sectioning, and to keep the window open and the temperature in the room below 50° Fahrenheit. The clear plate glass cover admits all the light needed for sectioning, but in setting the block a clearer view is obtained by reflecting the image from a small magnifying mirror.

As soon as a block is sectioned, the paraffine ribbon is lifted with a camel's hair brush to one of the cold slides in the freezer, the slide then carefully placed in one of the tin boxes and tightly covered. This is taken out of the freezer through the hand hole *H* and can be put in a cool place, fixing on the slide with the warm water method being deferred until all the blocks are sectioned.

After each block is cut, the next is immediately set for cutting, and the freezer again covered for ten or fifteen minutes. For during the process of sectioning the temperature in the operating compartment rises a few degrees, and the freezer must be kept covered until it has dropped again to 25° F. If while sectioning the knife becomes moist, the hands must be taken out at once, the hand-holes closed with the corks, and the freezer allowed to stand until the proper conditions of temperature are restored. Even the thinnest and longest ribbons do not snap or curl if the temperature in the operating compartment is not allowed to rise above the temperature of the knife.

In this way we cut from twenty-five to fifty blocks a day, all the material collected and imbedded during the summer requir-

large corks, held in place with two narrow tapes (shown in left opening). One of these tapes is passed through the ring of a screw in the center of the cork, and the tapes securely tied. If this is neglected the cold air in the freezer will force out the corks and cause a rapid rise of temperature in the operating compartment. *M*, drain tube from ice chamber. *N*, narrow air chamber, which is filled with cotton or some other non-conductor. *O*, copper handles. *P*, copper braces in ice chamber.

ing only a few days' work to prepare for study as needed. We always use very small blocks with four to six eggs arranged in a row in each block, and never more than one pair of ovaries in one block, as we find difficulties multiply with the increased size of the blocks, sections from the smaller blocks showing less tendency to curl or break.

It might seem that placing the microtome near an open window on a cold day would accomplish all we claim for the freezer, but the draught from an open window is sure to break the paraffine ribbon. Even in a cold room with closed windows, it is quite impossible to exclude currents of air, and the warm breath of the operator close to the microtome is a dangerous factor. In the freezer, the operating compartment is so nearly air tight that air currents are practically excluded. When the weather is intensely cold, we have sometimes used the freezer without ice, merely as a cover for the microtome, to enable us to section close to an open window, but we have always obtained the best results by using ice, and an even temperature as near 25° F. as possible.

For several years we have used this method of cutting thin sections of the egg of *Allolobophora*, for we had found it impossible to secure satisfactory thin sections of this egg with the methods in common use. Other investigators, however, rarely complain of difficulty in obtaining thin sections, and this may be due to fixing and hardening their material to a degree that makes any further hardening of the imbedded mass unnecessary, or to the fact that a large number of cells imbedded in one block of itself presents a harder mass than the single row of eggs we imbed in each block.

In our material these thin sections have been of the greatest value in aiding the interpretation of several obscure points; the constancy of the centrosome, for instance, was not demonstrated for this egg until we had secured sections of 3 μ or less. And for photographic reproduction at high magnification, thin sections offer very decided advantages, 3 μ representing the maximum thickness we have been able to use most successfully. It is possible of course to photograph the different planes of a thick[1] as well as a thin section, selecting the detail needed in each plane, but the structures above and below the selected plane being out of focus produce in thick sections a badly blurred image unfit for the best photographic reproduction.

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[1] With our method of focussing, a thick section requires a focussing (minus spherical) lens of lower power than the one used for thin sections.

Woods Hole, Mass.

HERMAPHRODITISM IN SABELLA MICROPHTHALAMA VERRILL.

LOUISE HOYT GREGORY.

In *Sabella microphthalama* Verrill, a paired, hermaphroditic sex organ appears in each segment of the body posterior to the pharynx. The study of this hermaphroditic condition was suggested by Professor Treadwell, who collected the material at Woods Hole, Massachusetts, in 1902–1903. The specimens were preserved in Hermann's fluid and stained in iron hæmatoxylin.

In general, the arrangement of organs as seen in cross section through the body is similar to that of other annelids. The paired sex organ is found on the dorsal side of the ventro-lateral bands of longitudinal muscles in the anterior end of each segment posterior to the setæ. It is supplied with blood from branches of the ventral blood vessel. Fig. 1 is a semi-diagrammatic draw-

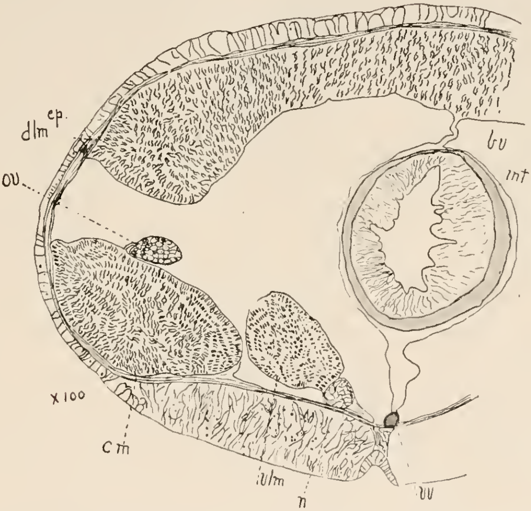

FIG. 1. Semi-diagrammatic cross section through the anterior portion of the body; *bv*, blood vessel; *cm*, circular muscles; *dlm*, dorsal longitudinal muscles; *ep*, epidermis; *int*, intestine; *n*, nerve cord; *ov*, ovary; *vlm*, ventral longitudinal muscle; *vv*, ventral blood vessel.

ing of one half of a cross section through a segment toward the anterior end of the body, showing the position of the sex organ in its relation to its surroundings.

Animals killed during the months of April, May, June, July and August differed from one another with respect to the form and contents of the sex organ, as well as to the condition of the body cavity.

All specimens killed in April and the early part of May were found to be pure females. Fig. 1 is a cross section through the body of a specimen killed April 27, showing the organ, which is a pure ovary. Fig. 2 is a magnified drawing of the ovary in Fig. 1. This is a typical organ found during the early months. It is a long, somewhat pear-shaped body, one end extending into the body cavity, the other end being attached by a band of tissue to the lateral muscles. At this same end is found the blood vessel. The cells are irregularly arranged and are in different stages of development; the majority of them contain very large nuclei with one or more nucleoli. In this organ there seems to be no definite arrangement of cells, large and small being intermingled. In the early part of April the organ is much smaller but it has the same characteristics as the one described. In none of these animals are the sex products found free in the body cavity.

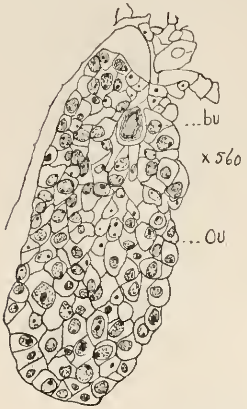

FIG. 2. Magnified drawing of ovary in Fig. 1. *bv*, blood vessel; *ov*, ova.

The animals killed during the months of May, June and July, with one exception, were hermaphrodites. During these months large masses of spermatozoa, and ova varying from the small oögonium to the large ovarian egg, were found free in the body cavity. Fig. 3 is a section through the body cavity of a specimen killed July 13, showing the different stages in the development of the ova as well as the masses of spermatozoa, both found free from the organ. *A* and *B* are sections through the outer surface of two large ovarian eggs. In the next section they have an appearance similar to that of *C*, *D* and *E*.

During August both hermaphrodites and females were found, the former being the more common.

Of the entire number examined, one third were distinctly female, and all but three of this number appeared among the specimens killed during April. No pure males were found in the material at my disposal. From these observations, it appears that the sexes are distinct at the beginning and end of the season, while during the middle of the season the sexes are united.

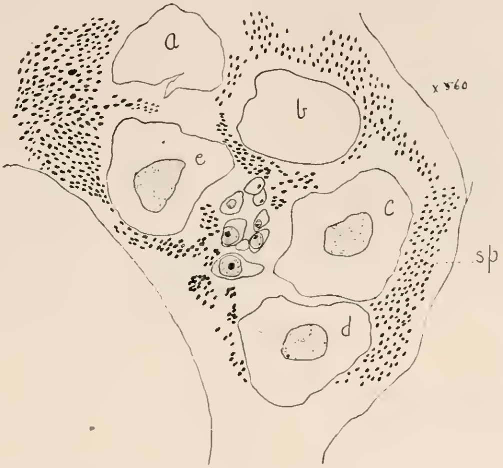

Fig. 3. Section through body cavity showing free ova and spermatozoa. *a*, *b*, *c*, *d*, ovarian eggs; *sp*, spermatozoa.

A typical hermaphroditic organ is seen in Fig. 4. It differs from the typical ovary in that it is shorter, broader, and more kidney shaped. Although ova and spermatazoa are found scattered throughout the organ, the majority of the ova appear massed together at the broader anterior end, while the larger number of male cells are found at the opposite end of the organ near the blood vessel. The ova are all in about the same stage of development. The cells on the very outer edge are ready to fall into the body cavity, where they undergo their further development. The male cells are in different stages of karyokinesis. In many cells the chromatin appears as a large, deeply stained mass, in some spindle shaped, in others in the form of an aster. In a few cells spermatozoa are found almost mature. Outside of the organ in the body cavity are seen cells still undeveloped, cells almost mature with a thin membrane about them, and cells entirely free. Together with these are found the ova. As a general rule the spermatozoa seem to pass through the greater part of their development in the organ before passing into the body cavity, while the ova fall into the body cavity in an immature condition and undergo the greater part of their development free from the organ.

In a great many cases, spermatozoa could not be found in the organ but were found in great masses in every segment. Ova filled the organ and were also free in the body. In other specimens spermatozoa were found in the posterior end of the body cavity only, while ova were found in the organ and free in the body cavity. From these observations it appears that the male products develop first in the organ, the development beginning at the anterior end of the body. In the cases where spermatozoa were found only in the body cavity, they had already developed in the organ and had become freed. In the cases where they were found only at the posterior end of the body, those at the anterior end had become mature and had passed entirely out of the body.

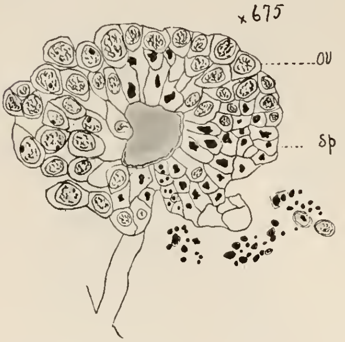

FIG. 4. Hermaphroditic organ. *Ov*, ova; *sp*, spermatozoa.

A comparison of these results with those found in *Ophryotrocha puerilis*[1] by Eugen Korschelt, shows many similarities as well as differences. In both annelids ova and sperm are found together in the same segment. In *Ophryotrocha* the sexual products are produced in a gland which has no definite structure, and which is merely a large sac in the body cavity. In *Sabella* the sex organ is more definite in outline and form. In this organ the sexual products are formed and develop until they pass into the body cavity where their appearance is similar to the appearance of the sexual gland in *Ophryotrocha*. In *Ophryotrocha* the sexual glands are found in about ten segments of the body. In the

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[1] Eugen Korschelt, "Ueber Ophryotrocha puerilis Clap. Metschn.," *Zeit. f. Wiss. Zool.*, 1893–94, Bd. 57, pp. 272.

anterior segments, the glands have male characteristics, in the central segments hermaphroditic characteristics, and in the posterior segments the glands appear to be pure ovaries. In *Sabella* the ova and spermatozoa are found together throughout the organ in every segment of the body posterior to the pharynx. In *Sabella*, no cells were found corresponding to the nurse cells of *Ophryotrocha.* They evidently do not appear as ova were observed in many stages of development. In *Ophryotrocha* pure males and females were found besides hermaphrodites having male characteristics yet containing ova, and those having female characteristics yet containing spermatozoa. *Sabella* shows only two conditions, that of a pure female and that of an hermaphrodite having both female and male characteristics. The specimens appearing to be pure females could not have been hermaphrodites with the spermatozoa already developed for in the case of the April 1st forms, the organ as well as the whole animal was too small to have already produced spermatozoa. Where the ovaries were large no spermatozoa or trace of them was found in the body cavity as would have been expected if they had already developed.

Somewhat similar conditions of hermaphroditism have been observed in *Hesione sicula*[1] by W. Bergemann. Here a long gland is found differing in shape from the sex organ of *Sabella* but producing both ova and spermatozoa. This gland is seen only between the sixth and sixteenth segments. It is not found throughout the body as in the case of *Sabella.* Nurse cells are also seen in connection with the ova which is unlike the condition of *Sabella.* Like *Hesione*, the male cells in the hermaphroditic organ of *Sabella* are generally nearer to the blood vessel as is seen in Fig. 4, and usually the more mature the sex products are, the nearer are they to the outside of the organ. Whether or not the organ is developed from the walls of the blood vessel as in the case of *Hesione*, could not be determined from the material at my disposal.

VASSAR COLLEGE,
May 30, 1905.

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[1] W. Bergemann, "Unterschungen uber die Eibildung bei Anneliden," *Zeit. f. Wiss. Zool.*, 1902, Bd. 73. "Ueber das spatere Schicksal der Zwitterdrusen von Hesione sicula," Zool. Anz., 1902–3, Bd. 26.

THE OSTEOLOGY OF CAULARCHUS MÆANDRICUS (GIRARD).

EDWIN CHAPIN STARKS.

The family Gobieosocidæ to which *Caularchus* belongs was at one time associated with the family Liparidæ on account of a ventral sucking disk possessed by both of them. Dr. Gunther[1] in 1861 worked out the anatomy of the disks, and showed that they were very different in structure. There being no other similarities between these two families the Gobieosocidæ has since that time been variously placed in the suborder Acanthopteri by different authors, as near the Blenniidæ, the Gobiidæ or the Batrachoididæ. Apparently the most rational disposition of the family has been made by Dr. Gill, who created for it the order Xenopterygii, which he placed with other orders of doubtful relationship with which it had nothing in common.

In undertaking the following work my interest was (1) to work over and describe the many osteological peculiarities of *Caularchus*, and (2) to attempt to find some indication of its relationship and systematic position. In the latter I have had small success, and I present this paper with reference to what anatomical value it may have.

The form I have chosen is *Caularchus mæandricus*, a species taken in abundance on the California coast. I have also examined *Gobiesox sanguineus* from the coast of Peru, and have added notes on its characters where they differ from those of *Caularchus*.

CRANIUM.

The cranium is much compressed and as wide as it is long. The vomerine region is broadly notched in front. On the superior surface and extending back to between the posterior margins of the orbits is a broad depression for the reception of the flat premaxillary processes. There is no supraoccipital crest and only very low temporal crests, which are connected across the

[1] Catalogue of Fishes of the British Museum, III., p. 495.

parietal region by a low transverse ridge. Long lateral processes from the prefrontal and sphenotic bound the orbital region. Broad wings from the sides of the parasphenoid form a wide floor anterior to, and in continuation with, the floor of the braincase. The basisphenoid and opisthotic are absent. There is no myodome. In *Gobiesox* the anterior portion of the cranium is scarcely depressed for the reception of the premaxillary processes.

The basioccipital is a flat bone not turned up at its lateral edges, and the greater part of it is covered by the wide parasphenoid. Its condyle is depressed, ellipitical in shape, and slightly inclined upward in opposition to the exoccipital condyles which are slightly inclined downward.

The exoccipitals are widely separated by the interposition of the basioccipital between them. They assist the latter bone in connecting the cranium with the vertebral column, presenting a round condyle at each side of the basioccipital condyle, so that the three parts of the basioccipital condyle are on a horizontal line.[1]

The surface of the supraoccipital is divided into two parts by the meeting of the parietals over its middle. The visible anterior portion of the supraoccipital thus separated from the posterior portion is nearly round in outline. In a few of the specimens examined the parietals just touch, in none of them do they meet broadly. In *Gobiesox* the parietals are well separated by the supraoccipital, extending over the edge of the latter only slightly.[2]

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[1] The condyles of the exoccipitals are wholly lateral to that of the basioccipital only in those fishes with a much depressed form, as *Callionymus*, *Remora* or *Caularchus*, though it does not at all follow that all fishes with depressed forms have lateral occipital condyles. It appears to be the rule that fishes with a depressed form never have the condyles of the exoccipitals wholly superior and in contact with each other, while those with a compressed form never have them wholly lateral.

[2] The condition of the meeting of the parietals should be more fully reported upon in other forms. Their union or separation by the supraoccipital has been used in the past in distinguishing large groups, and though the character has doubtless much less value than has been ascribed to it, its real value can not be known until it is more fully investigated. The difference between the condition of the parietals in *Caularchus* and *Gobiesox* is but a difference in degree where in one form they develop over the supraoccipital a little further than in the other and meet. This condition obviously has not the importance it has in some of the cyprinoid fishes where the parietals meet broadly entirely in front of the supraoccipital, and are the roof bones of the cranium in this region. The brief statement "parietals meeting" or "parietals separated by the supraoccipital" is far from being adequate. The condition of the parietals in *Caularchus* is not a very unusual one.

There is a notch on the posterior lateral surface of the epiotic formed between a short process above and the projecting lower edge of the epiotic below, into which the head of the posttemporal is received.

A long process projecting from the lateral edge of the pterotic passes along the posterior edge of the hyomandibular, and reaching almost to the opercle rigidly holds the suspensorium obliquely outward. A similar process formed by the sphenotic and the posterior end of the frontal projects over the anterior edge of the hyomandibular head, and forms the posterior orbital margin.

The frontals are broad and their anterior part as far back as the posterior margin of the eyes bears a large square depressed area in which the long flat premaxillary processes play.

The parasphenoid reaches its greatest width at its extreme anterior end where two wings reach far out on the prefrontals. It grows narrower below the orbital region, but again broadens in front of the proötics, and thence tapers quickly to a point at the posterior end.

The opposing proötics nearly meet at the median line above the parasphenoid, and in front of the basioccipital. The main parts of the fifth and seventh nerves occupy a notch in the anterior part of the proötic, but a small branch of the former runs through a small foramen just behind the notch.

The prefrontal is a wide bone projecting laterally in front of the orbital cavity, and supported behind by the broad parasphenoid. It is pierced near its center by the olfactory nerve.

The ethmoid is disk-shaped and apparently wholly membranous in origin. It is situated between the anterior ends of the frontals and does not extend in front of them.

The vomer is very broad, having a broad shallow notch in its anterior end, and with its lateral angles projecting. In a notch formed between each lateral angle of the vomer and the end of the palatine the maxillary fits just above its middle.

The Lateral Bones of the Head.

The hyomandibular, where it articulates with the cranium, terminates in two knobs, which fit into concavities in the sphenotic and pterotic. Below it has three articular processes; one for the opercle, one for the preopercle (by far the largest), and one divided between the symplectic and interhyal.

The symplectic is a slender bone bridging a large open space in front of the preopercle, and is the only bone between the hyomandibular and the quadrate in this region. It is received in a deep wedge-shaped notch, cut entirely to its tip, in the quadrate. The mesopterygoid and metapterygoid being absent the symplectic forms part of the anterior border of the cheek bones. In *Gobiesox* the symplectic runs behind the quadrate as usual, cutting from the latter only a small notch.

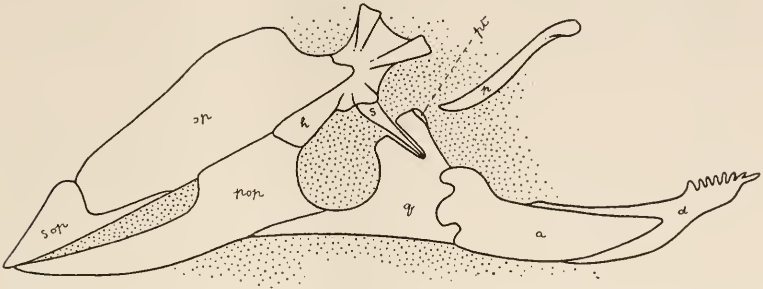

FIG. 1. Lateral bones of skull. *a*, articular; *d*, dentary; *h*, hyomandibular; *op*, opercle; *p*, palatine; *pt*, pterygoid; *pop*, preopercle; *q*, quadrate; *s*, symplectic; *sop*, subopercle.

The quadrate extends back covering the front of the preopercle. Above it is a very large open space, nearly circular in outline, bounded behind by the preopercle, and above and before by the hyomandibular and symplectic.

A small bone closely attached to the upper anterior edge of the quadrate is all that remains of the pterygoid. To it the palatine is attached by a ligament. It is very inconspicuous, and so closely united to the quadrate that it appears as a part of that element, but is easily separated in a macerated specimen.

The palatine is free from the other cheek bones, and is only connected to the reduced pterygoid by a ligament. Anteriorly it hooks over the maxillary.

The mesopterygoid and metapterygoid are entirely absent.

The articular is hinged to the quadrate by a very wide horizontal joint, making the jaw very rigid laterally. At the upper posterior angle is a strong process. There is no open space between the articular and the upper limb of the dentary.

The dentary is a strong bone and so closely attached to the articular that there is no play between them as is usual in other forms.

The angular is a very small nodule of bone on the inner surface of the articular, entirely hidden when the skull is viewed laterally. The interopercle is attached to the angular by a ligament.

The opercle, preopercle and subopercle together form an isosceles triangle with its greatest length extending backwards. The subopercle forms the posterior point of the triangle, and is interposed between the points of the opercle and preopercle. The preopercle is developed backward from its articulation with the hyomandibular to a long point, forming nearly the entire lower border of the opercular system. It forces itself between and entirely separates the subopercle and interopercle, bringing the opercle entirely above it. Its anterior end extends forward in a long point behind the quadrate nearly to the mandibular condyle. The interopercle is a small triangular bone, lying free behind the quadrate and hidden from sight by that bone when the skull is viewed laterally. It is unconnected except by a ligament to the angular. There is a low ridge running along the lower edge of the opercle, and a very high sharp one running along the lower edge of the preopercle and the quadrate nearly to the condyle of the mandible.

A broad membranous preorbital plate is all that remains of the suborbital chain of membrane bones. It bears a sensory tube which opens anteriorly to the exterior through a small pore a couple of millimeters in front of the anterior nostril. The tube crosses the plate obliquely downward and backward, forking widely in front of the eye, and opening to the exterior at the lower edge of the plate through two widely separated pores. These pores appear in the skin at the upper border of the mouth 6 or 7 mm. apart.[1] A third pore 4 mm. anterior to these, and near the front of the mouth, forms the exit of the sensory tube traversing the nasal plate.[2] At the place the preorbital tube forks the plate is slightly ossified.

[1] The specimen described is 1 cm. in length.

[2] It appears probable that the suborbital sensory tube is never present in the Teleosts unossified. There may be a series of pores or nerve hillocks, as in *Porichthys*, but they do not open into a continuous tube. I have examined a large number of forms known to have no suborbital bones, but find no tube present in any of them.

The maxillary elements are very strong. The premaxillaries send back long wide processes over the top of the head to between the eyes. Each maxillary bears a notch on its upper surface for the reception of the edge of the premaxillary process. The notch is some distance from the head of the maxillary and allows it to meet its opposite fellow below the premaxillary processes, but viewed from above the maxillaries appear to be widely separated. There is a depression in the upper surface of each maxillary into which the palatine fits.

The nasals are wide bones meeting on the median line, covering the anterior part of the premaxillary processes, and interposed between the maxillaries above.

THE BRANCHIAL AND HYOID BONES.

The basibranchials are entirely absent, and the hypobranchials are but slightly separated at the median line. The latter are all of the same length, very long and much constricted at the middle. They appear to be entirely cartilaginous in a fresh skeleton, but as they dry a fine hard calcareous matter becomes evident. The hypobranchial of the fourth arch is missing, as in all other teleosts (so far as known). The ceratobranchial of the fourth arch is as long as the combined length of the ceratobranchial and the hypobranchial of the third arch,[1] and opposite the union of these two elements it is angulated, so that its lower part has the appearance of being the fourth hypobranchial. The superior pharyngeals of each side are anchylosed into a single tooth-bearing bone. The inferior pharyngeals are in contact only at their anterior ends.

A very small nodule of bone between the hypohyals on the lower surface represents the urohyal, and another on the upper surface the glossohyal. Only one of the hypohyal elements is present on each side. The suture between the hypohyal and the ceratohyal runs vertically upward from the lower side of the arch half way across the arch, then turns squarely and runs horizontally backward for nearly half the length of the ceratohyal, where it again turns upward and reaches the upper side of the arch. The other hyoid elements are normal. Six branchiostegal rays are present; two are attached to the outer surface of the epihyal, two to the outer surface of the ceratohyal, and two to the inner surface of the ceratohyal. In *Gobiesox* the urohyal and glossohyal are better developed, the former being scarcely reduced.

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[1] I am not aware of any similar arrangement in any other Teleost. The hypobrachials usually decrease in length so rapidly posteriorly that their absence on the fourth arch does not require a lengthening of the fourth ceratobranchial; all of the ceratobranchials being of about the same length.

The Shoulder and Pelvic Girdles.

The posttemporal is a long simple ray of bone inclined obliquely outward and forward from its attachment with the cranium, so that its outer end is farther forward than its inner. It thus forms a sharp angle with the supraclavicle, which is inclined forward as usual. In place of the usual lower limb a long ligament connects it with the inner edge of the exoccipital near the basioccipital. Its proximal end is not very firmly attached to the cranium. A short epiotic process projects over its head, and

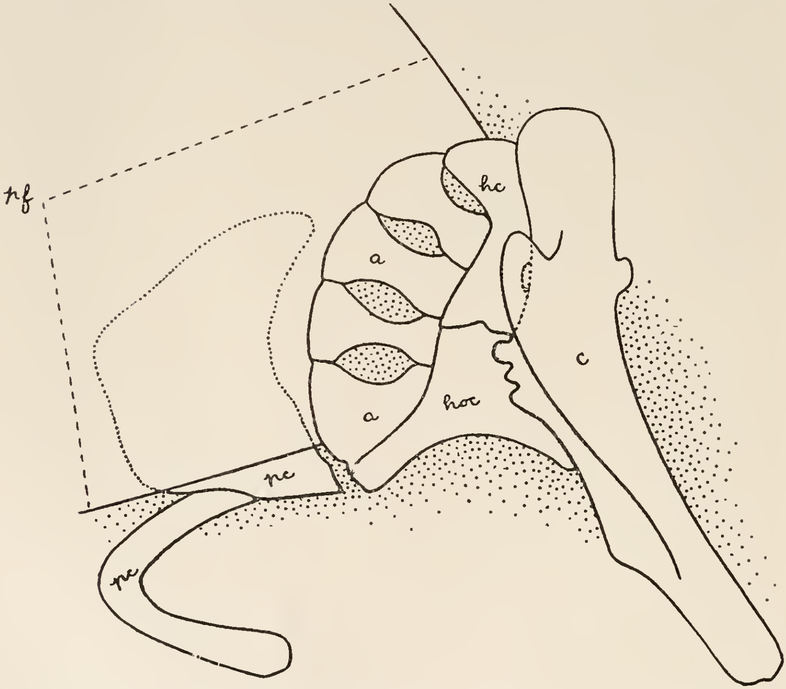

Fig. 2. Shoulder girdle. *a*, actinosts; *c*, clavicle; *hc*, hypercoracoid; *hoc*, hypocoracoid; *pf*, pectoral fin; *pc*, post clavicle.

two stout ligaments are attached to it, one above and one below, holding it firmly against the epiotic, but leaving it movable laterally.

The supraclavicle is a long simple bone. At its lower end is a concavity which fits over a knob on the clavicle.

The clavicle is slightly bent at an angle near its middle. From its anterior edge a large lateral wing projects outward and backward. A short distance above its middle on its anterior edge is a tubercle of bone over which the cup-shaped end of the supraclavicle is attached.

The hypercoracoid is constricted at its middle opposite the base of the upper actinost so that it appears as a fifth actinost.[1] This illusion is enhanced by the pectoral fin being joined to it by two or three rays. It lies along the entire length of the upper actinost, but is separated from it, except at each end, by an open space similar to that between the actinosts. As the clavicle overlaps the hypercoracoid to the edge of the hypercoracoid foramen the foramen appears to be between these two bones. It is, however, through the middle of the hypercoracoid.

The hypocoracoid is joined as usual to the clavicle and hypercoracoid but its lower end instead of returning to the lower end of the clavicle, as usual in most fishes, projects backward along the lower edge of the lowest actinost. This condition is evidently brought about by the position of the pelvic girdle which is closely attached along the inner lower edge of the clavicle.

The actinosts are hour-glass-shaped, four in number, and separated from each other by open spaces. The coracoid elements each support two actinosts.

The postclavicle has lost its attachment with the clavicle and lies free opposite the base of the pectoral fin just behind the pelvic girdle. It is divided into two parts. The inferior portion is curved around the outer and posterior portions of the ventral disk, and the posterior fringe of the disk is attached to its edge. The superior portion is very broad and from its posterior edge is developed the peculiar fin-like flap seen externally on the fish just behind the pectoral fin. In *Gobiesox* a flat process is developed forward from the upper element of the postclavicle to the upper end of the clavicle.

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[1] In the accompanying drawing this loses its deceptive appearance on account of the exaggeration of the distinctness of the sutures.

The pelvic girdle is closely attached along the posterior lower edge of the clavicle. The opposite sides are suturally attached along the median line, and together send back a long stout median spine through the middle of the ventral disk. Toward the anterior end each side bears a large foramen. The ventral fins are attached to the outer edges of the girdle, with their bases directed transversely across the body, or towards each other, rather than being attached, as in other forms, to the posterior ends of the girdle with their bases directed forward.

Vertebral, Rib and Fin Elements.

The abdominal vertebræ number 13, the caudal 19, making with the hypural a total of 33.

The first vertebra is expanded laterally to accommodate the wide occipital condyle. It bears no ribs. The abdominal vertebræ as viewed from below are smooth, without longitudinal ridges or pits, and are much constricted at the middle. On the side each bears a deep pit for the reception of the head of the rib. The last two abdominal vertebræ bear small parapophyses to the under side of the base of which ribs are attached. The base of each abdominal neural arch is expanded laterally over the head of each rib into a horizontal wing-like process, which grows small and disappears posteriorly. Anteriorly each projects in a point at the side of the preceding neural process. These processes are doubtless zygopophyses, though they might be considered parapophyses, as the ribs are expanded upward to their lower surface, were it not for the small processes on the last abdominal vertebræ which, however, prove themselves to be true parapophyses by passing into the hæmapophyses posteriorly· The caudal vertebræ grow more compressed backwards, and exhibit no peculiarities. In *Gobiesox* the vertebral formula is as follows: 14 + 21 + 1 = 36. The zygopophyses project laterally as in *Caularchus* but áre not expanded.

The hypural is symmetrical having a longitudinal incision dividing it into equal parts, each part bearing an equal number of caudal rays. There is no urostyle or other indication of heterocercy. The spines of one vertebra anterior to the hypural assist in supporting the caudal fin.

Stout ribs are present on all of the abdominal vertebræ except the first one, and are continued back on the anterior caudal vertebræ. They are vertically flattened and grow smaller posteriorly. They project horizontally outward and to the lower surface near the tip of each is a bony ray which curves downward around the abdominal cavity. Gunther[1] describes these as follows: "The epipleurals are not much less developed than the ribs to the extremities of which they are suspended. We might also consider the ribs as long and detached parapophyses, and the epipleurals as the ribs proper." The presence of normal parapophyses on the last abdominal vertebræ prove the latter supposition to be incorrect, and if the distal bones are epipleurals they have changed their usual position on the superior surface near the bases of the ribs to the inferior surface near the tips of the ribs. The only other possibility is that they are extra intermuscular ossifications as occur in some fishes, notably the clupeoid fishes.

No trace of a spinous dorsal remains, and there are no accessory interneural rays in front of the soft dorsal. There are four unbranched ventral rays, and a short flat bone hidden under the skin in front of them representing a ventral spine, the rays are thick and their cross articulations are very close as in the thickened lower pectoral rays of the Cottoid fishes. I do not find in either *Gobiesox* or *Caularchus* the free ventral ray that Dr. Gunther found in *Chorisochismus dentex*.

The interspinal elements are normal in arrangement, and exactly coincide in number with the vertebral spines to which they are attached. No bony baseosts are developed.

RELATIONSHIPS.

The structure of the skeleton gives little help in assigning to the family Gobiesocidæ a definite phylogenetic position, and I find myself no nearer to a solution of the problem than Dr. Gill was when he wrote of these fishes in the "Standard Natural History," (Vol. III., p. 267) as follows: "There are no spines in any of the fins, and thus it has not even the technical characteristics of the true Acanthopterygians. Doubtless the parentage of the stock is to be looked for in that great suborder; but the divergence of the known forms has been so great that at present it cannot be certainly predicated whereabouts to find it."

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[1] Cat. Fish. Brit. Mus., III., p. 493.

I have examined skeletons of Cottoid, Blennioid, and Gobioid fishes with small results. The families Batrachididæ and Callionymidæ offer some slight indications of relationship to the Gobiesocidæ, and the weight of evidence is thrown towards the former family by the young of some or all of them having a ventral sucking disk just behind the base of the pectorals. The family Batrachididæ[1] further resembles the Gobiesocidæ in having the suborbital ring reduced to a small preorbital bone, only very small parapophyses present posteriorly, no myodome, and a single superior pharyngeal present on each side. As opposing the idea of relationship the Batrachididæ have five long actinosts, the posttemporal forms an integral part of the cranium, the palatine is normally joined to the pterygoid, and the mesopterygoid, metapterygoid, alisphenoid, and basibranchials are present.

The family Callynomidæ[2] resembles the Gobiesocidæ in having no mesopterygoid or metapterygoid, thus leaving the symplectic to form part of the anterior border of the cheek bones, in having no myodome or suborbitals, in the ventrals being widely separated, as well as in the general form of the body. The Callionymidæ, however, possess some important and well marked characters not possessed by the Gobiesocidæ, and these probably more than counterbalance the characters held in common. These characters are briefly: a spinous dorsal present; the ethmoid extending back and forming a bony interocular septum; the frontals reduced and occupying little more than the interorbital space; the posttemporal forming an integral part of the cranium; the actinosts all abutting against the hypocoracoid; the hypercoracoid foramen between the coracoid elements cutting an equal notch from each; the palato-quadrate arch normal; three superior pharyngeals present on each side; basibranchials present; the neuropophyses and hæmopophyses ending each in two spines between which the interspinous elements fit.

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[1] In this family the following genera were examined: *Batrachoides*, *Opsanus* and *Porichthys*.

[2] *Calleonymus* was the only genus examined.

SYNOPSIS OF CHARACTERS OF THE FAMILY GOBIESOCIDÆ.

Body broad and depressed in front, covered with smooth naked skin. Premaxillaries protractile. Strong teeth on dentary and premaxillary; none on vomer or palatines; pseudobranchiæ small or wanting. Gill fringes on two and a half or three arches. Occipital condyle partly formed by exoccipitals, which present articulating surfaces entirely lateral to that of the basioccipital. No supraoccipital crest present. Parietals separate or meeting over the surface of the supraoccipital. Myodome absent. No basisphenoid, alisphenoid, opisthotic, mesopterygoid or metapterygoid. Pterygoid reduced. Palatine connected to pterygoid only by a ligament. Preopercle developed backwards in a long triangular process, interposed between, and widely separating the interopercle from the subopercle. No suborbital ring. Basibranchials absent. Fourth ceratobranchial much lengthened. Superior pharyngeals one on each side. Hypohyal single on each side. Six branchiostegal rays. Posttemporal a single ray of bone without a lower limb. Supraclavicle attached just above middle of clavicle. Hypercoracoid foramen through middle of hypercoracoid. Actinosts hour-glass-shaped; two attached to each coracoid element. Postclavicle in two parts; the inferior part supporting the posterior edge of the ventral disk. Spinous dorsal absent. Ventrals each with a concealed spine and four unbranched rays. No airbladder. Parapophyses developed only posteriorly. Zygopophyses produced laterally. A ray of bone attached to each rib extending down around abdominal cavity.

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THE CHROMOSOME COMPLEX OF ORTHOPTERAN SPERMATOCYTES.

C. E. McCLUNG.[1]

A subject of perennial interest is offered by the maturing germ cells, and that appreciation is not lacking is well evidenced by the large annual output of papers devoted to different phases of the question. The task of keeping abreast of this literature has become a considerable one, especially since it is now necessary to take into account the investigations upon hybrid matings and upon unusual or modified methods of fertilization. Despite regret at the increased labor thus brought about, one cannot but rejoice at the enlarged conceptions of chromosome functions which have followed from this union of two apparently different lines of investigation.

It cannot be gainsaid, I think, that as our knowledge increases it becomes more and more evident that in the chromosomes we are dealing with intracellular elements of definite morphological character which are self-perpetuating and which have to do with the development of precise characters in the organism of which they are a part. In my early study of the male germ cells of insects I became convinced of this individuality of the chromosomes, and in all my papers I have emphasized this conception and have brought forward proof in support of my position. ' It is a pleasure to acknowledge here the material assistance that has been rendered me in this endeavor by the consistent results of my students. Realizing, however, the extensive character of the problem, I have confined the work to a somewhat limited field, but within this have made broad comparative studies. As the work progressed there was indicated the prevalence of a general plan of chromosome structure throughout the tracheate Arthropoda studied and it then became necessary to undertake careful researches within smaller groups, some of which have

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[1] I am much indebted for assistance in carrying out work on Orthopteran germ cells to the Carnegie Institution, which made grant No. 16 for this purpose.

already been embodied in papers devoted to the Orthopteran families.

During the course of these investigations it became plain that there were variations from the type of maturation mitoses, and that these afforded facts which seemed to indicate that it might be possible to discover some correlation between individual chromosomes and body characters. It is the purpose of this paper to bring forward some of these facts and to suggest conclusions arising from our thus increased knowledge of the maturation chromosomes. In venturing these deductions I have had in mind their application to the particular forms studied, but at all times have tried to bring them into relation with facts derived from the study of other organisms. This, I believe, is justifiable in the present state of our knowledge, for it is done on the supposition that reproduction is, in `the main, the same process throughout the range of organic forms. With the accumulation of observations the conviction is borne in upon us that the maturation mitoses of all organisms are, in general, of one pattern, and that the burden of proof lies upon him who would argue for individual types.

There is always the danger, to be sure, that we have not reached down to the basic facts, but are conceiving more superficial ones to be all important. This is, I imagine, unavoidable in the preliminary stages of an investigation, but it can lead to no harm if the facts in each case have been accurately determined. In the absence, therefore, of definite knowledge of the chromosomes in the germ cells of organisms exhibiting Mendelian characters or mutations we are warranted in supposing them to be of the same general character as the ones known until they are proved different.

The present paper is one of a series in which it is hoped to develop the history of the maturation chromosomes in the Orthoptera. Obviously this is a task of great magnitude and no far-reaching conclusions can be attained until a large series of forms has been studied. Already a not inconsiderable number has been worked over, and each year additions are made. Recently I have been devoting myself again to the family Acrididæ, and in some newly observed forms I have encountered certain peculiarities of chromosome structure which seem to me of considerable importance. Some of these will here be considered.

It will hardly be necessary to enter into any account of the chromosomes in the Acridian family since this subject has already been discussed in a former paper ('00). Familiarity with the spermatogenesis of these Orthoptera will, therefore, be assumed and only such details gone over as are new or, at present, better understood.

The forms upon which the new observations were principally made are three species of the Acridiinæan genus *Hesperotettix*, and a species of *Mermiria*, a Tryxaline. While the facts stated have to do with these forms, they derive their importance from their relation to what appear to be common processes in the groups to which they belong. Much more work is, therefore, involved in the attainment of these results than would appear on first thought, and the basis for theories is correspondingly widened. In addition to the facts gained from the species mentioned, confirmatory evidence on a number of points afforded by some incomplete observations on *Chortophaga viridifasciata*, an Acridian, and *Anabrus*, a Locustid

II. Observations.

1. *Number of Chromosomes in the Family Acrididæ.*

It may be recalled that in previous papers I have not laid much stress upon reported numbers of chromosomes, because of the difficulties involved in securing accurate enumerations. Nevertheless the full importance of this knowledge has not lacked appreciation, and latterly more attention has been directed toward the determination of the number of chromosomes in the different species of Acrididæ. In order to avoid the operation of the personal factor as much as possible, camera lucida drawings were made in considerable numbers from time to time and laid away. Finally the drawings were taken and the counts tabulated. As only the clearest cases were used for drawing, and as every precaution was taken to see that the entire complement of chromosomes for each cell was present, it is thought that the figures are reasonably accurate. Since this is the judgment of one who has at all times been decidedly critical toward the subject of chromosome enumerations, it may lend assurance to the results.

Regarding the enumerations I would say that much confidence is reposed in those of the spermatocyte elements, but that there is less certainty attending the figures derived from the spermatogonia. This is due to the fact that the latter cells are relatively small and the chromosomes very large, so that the elements are not well separated and clear. There is no fusion of the chromosomes in the metaphase, but they are long and sinuous and their limits hard to discern.

In general, then, it was found that for the family the number of chromosomes in the spermatogonia is 23 and in the first spermatocyte 12. Exceptions were found in *Hesperotettix* which has 23 in the spermatogonia and 11 in the first spermatocyte; and in *Mermiria* which has 23 in the spermatogonia and 10 in the first spermatocyte. Careful study revealed the fact that the exceptions are only apparent, and that the reason for the deviation from the family character is due to unusual associations of the spermatogonial chromosomes in the spermatocytes. This is brought about by the action of the accessory chromosome which here gives further evidence of its highly important character. It will be well to outline the history of the chromosomes in these genera separately and then refer to the principles of association involved later.

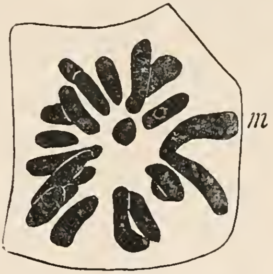

FIG. 1. Polar view of a spermatogonium of *Hesperotettix speciosus* (all the drawings of this genus were made from *speciosus* material) in metaphase, showing a tetrad "*m*." Not all the chromosomes are drawn.

2. *The Chromosomes of Hesperotettix speciosus.*

It is not my intention to discuss the whole group of chromosomes in this species, but merely to give the history of the most strongly marked element. I hope subsequently to make a careful detailed study of the germ cells of the various species of this genus to establish the relations of the entire complement of chromosomes, but at present that is not possible.

If we examine the spermatogonial mitoses of this species we find that the chromosomes are of the usual rod-shape type, but among them there occurs one that is peculiar in being bivalent instead of univalent. The chromatids composing this multiple chromosome[1] are amongst the largest in the cell and are easily distinguishable. During the anaphase it is seen that the multiple chromosome exhibits the so-called heterotypical form of division, and the figure produced is exactly the same as that resulting from the division of the tetrads in the first spermatocyte. In reality we have here a tetrad that is in every respect the same as those in the succeeding generation. A precocious synapsis of chromosomes in the spermatogonia is responsible for the unusual type of division in this generation of cells.

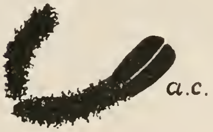

FIG. 2. The hexad multiple chromosome of *Hesperotettix* in the first spermatocyte prophase. The accessory chromosome "*a.c.*" is homogeneous and the ordinary chromosomes granular, as is usually the case for these elements at this stage.

There can be no mistake about the nature of this multiple chromosome, for the line of fusion between the two chromosomes is clearly marked, and the mantle fibers of the metaphase attach at the center of the U-shaped element. In the anaphase the contrast between this chromosome and the others is very marked, for the daughter chromosomes of the latter ascend to the poles as straight rods with the fibers at the ends, while the former are U-shaped and have the fibers annectent at the center, or where the chromatids are joined together.

The early prophase of the first spermatocyte shows this bivalent element among the others, which have by this time also become bivalent, but it may still be distinguished by its greater length. When the chromosomes commence to shorten and condense the accessory chromosome attaches itself to one end of the large bivalent element, thus forming a trivalent chromosome. As thus constituted the multiple chromosome is much longer than any of the others, and clearly shows the limits of the three parts. A very peculiar condition prevails at this time. As is usually the case in the prophase, the ordinary chromosomes are granular and irregular in outline, while in striking contrast the accessory end of the multiple chromosome is homogeneous with sharply cut boundaries. The trivalent chromosome, or hexad, thus exhibits a very unusual appearance, because of the heterogeneous condition of its parts. Besides this structural difference, the accessory chromosome is further made noteworthy by the fact that it is shorter than the other thirds of the multiple element.

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[1] I shall use the term "multiple chromosome" for all elements containing more than two chromatids.

When the mitotic figure of the first spermatocyte is formed the hexad takes a position parallel to the axis of the spindle with the division between the parts of the original bivalent chromosome in the equatorial plate. As it lies thus it is almost as long as the spindle, but has approximately two thirds of its length on one side of the equatorial plate. This greater portion includes, of course, the accessory chromosome. Since the fibers always attach to the ends of the chromosomes, the accessory becomes bent away from the spindle and lies at a more or less obtuse angle to the rest of the chromosome. The mantle fiber then attaches to the angle thus established. A similar arrangement is mentioned by de Sinéty for the phasmid *Leptynia.*

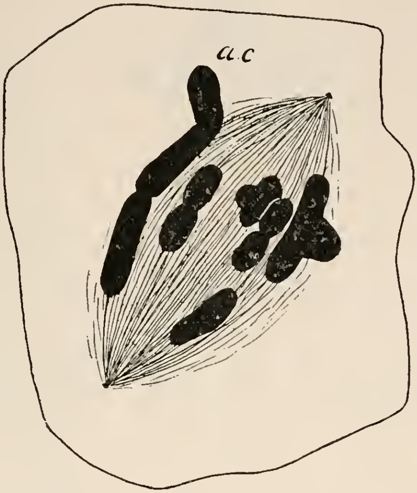

Fig. 3. Lateral view of the first spermatocyte prophase with a number of thechromosomes drawn. The hexad is shown in profile with the accessory chromosome, "*a.c.*," characteristically bent back at an angle. These, and all other drawings in this paper, were made under a camera lucida and are reproduced here at a magnification of 1,900 diameters.

With the beginning of the anaphase this multiple chromosome separates at the point where the two chromosomes were united in the spermatogonia, so that to each end of the spindle there goes one of the spermatogonial elements, but the accessory chromosome, on the contrary, becomes a member of only one of the daughter nuclei. Thus it happens that in the first spermatocyte where the quantitative division of the other chromosomes occurs this one element is divided qualitatively. In effect this is also a qualitative division of the accessory chromosome which here exhibits the same behavior as has been described for the Locustids, the difference being that in the case of *Hesperotettix* there is a close association between the accessory and an ordinary tetrad. It would seem that there is here an attempt on the part of the accessory chromosome to establish the usual relationships that are formed between chromosomes at this period of the maturation process. To this extent the element departs from what has been regarded as one of its most characteristic features, that of exclusiveness. As a matter of fact, however, all the later work upon this structure has tended to show similarities to ordinary chromosomes rather than differences from them, and we are not surprised, therefore, to find it uniting itself with another chromosome in the ordinary manner. Being the odd member of the group it is unable to accomplish this step in the usual way and is therefore forced to become a member of a hexad element.

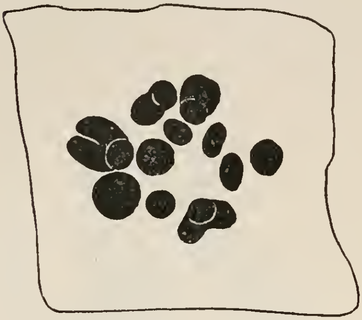

Fig. 4. Polar view of first spermatocyte metaphase, *Hesperotettix*, showing all the chromosomes of the complex.

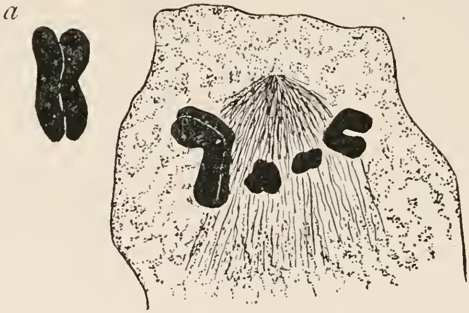

Fig. 5. Lateral view of one pole of first spermatocyte anaphase, *Hesperotettix*, with four chromatic elements drawn. The multiple chromosome at the left consists of the accessory chromosome and one half of the tetrad to which it was united. The relative positions are the same as in Fig. 3. Fig. 5*a* represents such a multiple chromosome *en face*.

Why it should always be the same tetrad that is selected for this association is not clear, and will perhaps become apparent only when we learn the meaning that attaches to the intimate association among the maturation chromosomes.

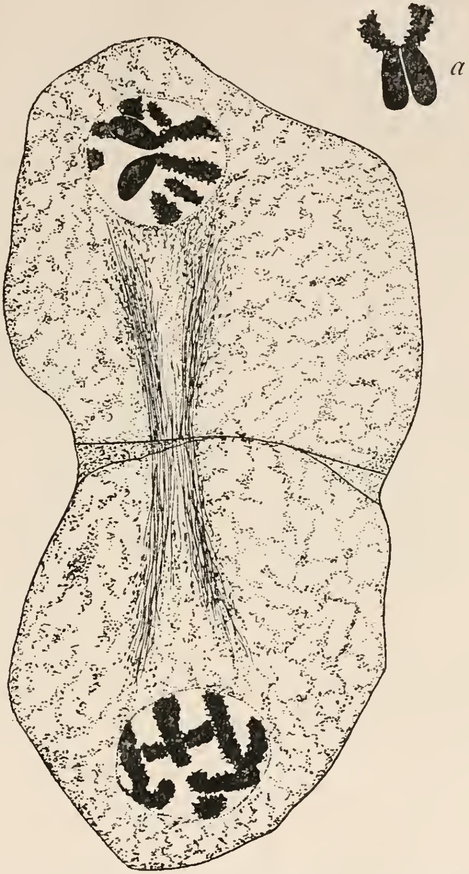

FIG. 6. Telophase of first spermatocyte of *Hesperotettix*. At the upper pole is the multiple chromosome in which the accessory chromosome remains homogeneous while the other element of the tetrad has again become granular. Homologous chromatids are well separated. Fig. 6*a* shows a similar chromosome with the accessory chromatids less divergent.

The close connection thus established by the accessory chromosome persists through the later periods of the maturation process. In the telophase the pair of chromosomes, consisting of the accessory and the half of the tetrad, very much resembles the ordinary chromatin elements of the prophase, being, as it is, tetrad in character. Here as always, however, the accessory remains homogeneous while the associated chromosome becomes granular like its fellows. With the establishment of the second spermatocyte mitotic figure this oddly constituted tetrad takes its place in the equatorial plate with the mantle fiber attached at the point where the accessory chromosome is joined to the other one. Since the accessory is the shorter the fiber is not inserted at the middle of the chromatin rod but slightly to one side. The normal form and position of the chromosomes of the second spermatocyte in metaphase is that of a split rod pointing radially away from the axis of the spindle with the chromatids placed one above the other in the plane of the spindle axis. While all of the remaining chromosomes of *Hesperotettix* are thus arranged, the accessory chromosome and its mate are bent upon each other so as to present an angle to the spindle. It thus happens that in the anaphase there is apparent a heterotypical division of the multiple chromosome in which the resulting V-shaped loops each have one arm shorter than the other. It is now evident that the chromosome associated with the accessory has taken part in a heterotypical division in the spermatogonia and in the second spermatocyte, and in each case the plane of division corresponds to the original longitudinal cleft of the spireme thread. Because of the early association between the accessory and the tetrad there can be no doubt regarding the planes of division in the hexad.

I have not as yet attempted to work out in detail the specific characters of the *Hesperotettix* chromosomes, although I have that in mind for an early investigation. But even in the preliminary study here presented there have become apparent specific differences in the first spermatocytes which seem to be constant and which I will briefly notice. The description given above is based upon *Hesperotettix speciosus*, a form of larger size than the others and with strongly marked characters. Besides this there are in the same habitat two other species that are somewhat common and which are plainly different from *H. speciosus* but resemble each other in size and general appearance. This close resemblance caused my collectors to confuse the material from which slides were made, and so I am not sure whether I have *H. pratensis* or *H. viridis.* I am tolerably sure that both are represented, although I have not as yet been able to detect differences enough in the cells to separate the preparations into two groups.

Despite the uncertainty regarding the exact character of the material, it is clear enough that it is not from *H. speciosus*, so that we know that any differences manifest in the cells will be between *speciosus*, definitely identified and described above, and either *viridis* or *pratensis.* From this comparison it is interesting to find that there are constant differences in the germ cells which are correlated with constant differences in body structures. So far I have made no attempt to trace variations beyond those shown by the hexad multiple chromosome. Even here only a start has been made, but it will suffice for my present purpose merely to show that there are differences between species of a genus in germ cell architecture.

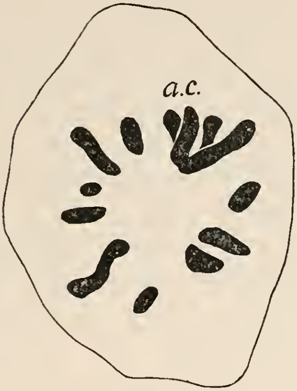

FIG. 7. A polar view of the second spermatocyte of *Hesperotettix* containing the accessory chromosome "*a.c.*" It forms the smaller half of the tetrad. Not all the chromosomes present.

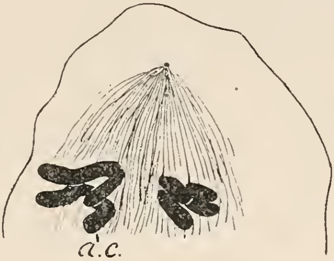

FIG. 8. Fragment of a second spermatocyte of *Hesperotettix* in metaphase showing the tetrad containing the accessory chromosome and two ordinary chromosomes.

First we must notice that there are present in both species multiple chromosomes of the same sort. This must be regarded as a generic character, and for specific differences it will be necessary to look for modifications in size and proportions of parts. In drawings made at a magnification of 2,875 diameters it is found that the accessory chromosome of *H. speciosus* in metaphase measures on an average about 12 mm. while in *H. viridis* (?) it is near 11 mm. This difference is only slight, but if the chromosomes associated with the accessory are measured it will be found that the variation in size is considerable. Thus in *H. speciosus* the average length is in the neighborhood of 20 mm. while in *H. viridis* it is not over 15 mm. Here is a very pronounced specific variation which is probably accompanied by corresponding variations in the other chromosomes. Along with this difference in size of the chromosomes there goes a corresponding variation in the achromatic figure. The spindle in the first spermatocyte of *H. speciosus* is long and slender, while in *H. viridis* (?) it is short and heavy. There are probably many other less obvious differences which I have not discovered in this preliminary survey. I hope in time to present these in detail.

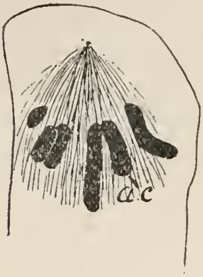

FIG. 9. A similar cell in anaphase. The disproportion between the accessory part of the dyad and the other member is well exhibited.

3. *The Chromosomes of Mermiria sp.*

In the spermatogonia of *Mermiria* there is again found the heterotypical mitosis of chromosomes. The early synapsis of chromosomes, which is the occasion for the unusual form of mitosis, is encountered very frequently in spermatogonia, and I shall have occasion to refer later to instances in certain species where it is very marked. In the prophase of the first spermatocyte of *Mermiria*, there is again found a multiple chromosome which shows the same constitution as the one in *Hesperotettix*. The pronounced difference between the smooth homogeneous accessory chromosome and the rough granular elements of the the tetrad joined to it is strikingly shown in the beautifully clear nuclei of this Tryxaline. So far the case is parallel to that exhibited by *Hesperotettix*, but just before the metaphase a singular and entirely unique association of the hexad multiple chromosome of the prophase with one of the tetrads occurs. This is brought about by the end to end union of the parts which produces a pentivalent chromosome, or decad. So far as I know no such a chromosome as this has ever before been described.

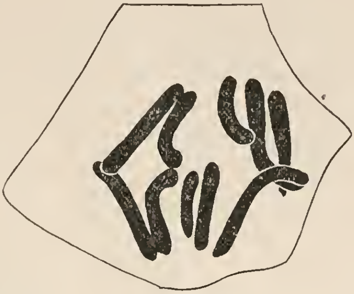

Fig. 10. Lateral view of some of the chromosomes in the metaphase of a spermatogonium of *Mermiria*. The multiple chromosome shows its tetrad character well.

The metaphase exhibits this strikingly peculiar chromosome extended along the length of the spindle parallel to its axis. The terminal parts are variously placed, sometimes being bent back parallel with the middle portion, sometimes extending out at right angles to it, and again being directed upward at an obtuse angle. Frequently the two terminal elements are differently inclined to the middle portion. In any event the archoplasmic fibers attach, not at the free ends of these chromosomes, but where their other ends join the main shaft of the element. One point which I have not yet been able to determine with certainty is the position of the accessory

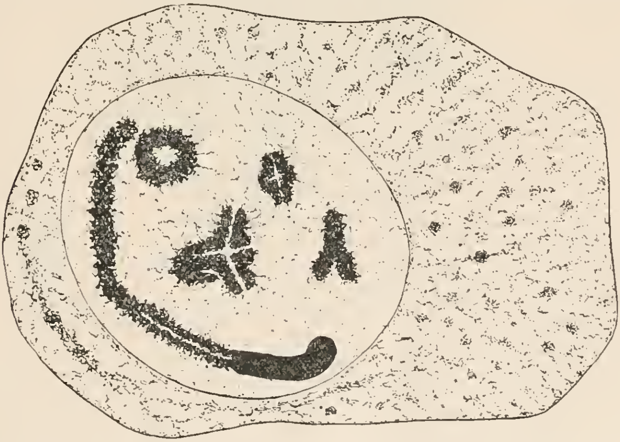

Fig. 11. Prophase of first spermatocyte of *Mermiria* showing particularly the accessory chromosome attached to a tetrad as in *Hesperotettix*. The organization of the cytoplasm is also indicated.

chromosome in the multiple element. Whether the tetrad that is added to the multiple chromosome attaches to the accessory end of the trivalent element of the prophase, or to the other extremity, I have not made out with certainty. The final result of the divisions would not be different in either case.

Upon the separation of the chromosomes in the metaphase the multiple chromosome is divided so that to one pole there goes a trivalent element and to the other a bivalent one, the difference in valence being due to the presence of the accessory chromosome in one daughter cell. There occurs here an entirely unique separation of chromosomes, for by means of it *entire tetrads pass into the second spermatocytes.* If these bivalent structures are always produced by the fusion of paternal and maternal chromsomes, then in this case both such pass into one cell. The alternative possibility that they are not homologous chromosomes will be discussed in the latter part of this paper.

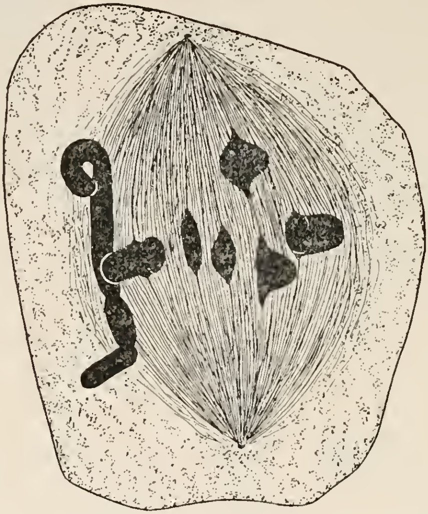

FIG. 12. Metaphase of first spermatocyte in *Mermiria* showing the decad in profile and a number of the tetrads. Observe that the fibers attach at the point of contact between two chromosomes and not at the free ends of the chromosomes. A very large part of the cytoplasm is organized into the spindle and the remainder of it is measurably polarized.

When the second spermatocyte mitotic figure is formed it is seen that the accessory has separated from the dyad with which it was united and has divided longitudinally as usual. But besides the accessory and the ordinary dyads of the second spermatocyte, there is present in each cell a tetrad which was a part of the multiple chromosome and which did not undergo division in the first spermatocyte. This may be found occupying on the second spermatocyte spindle such a position as tetrads usually occupy in the first spermatocyte, and under these circumstances it divides in the customary longitudinal manner. Here again there is a heterotypical mitosis along with the ordinary type, a condition which was also present in the spermatogonia. In both cases there is indisputable evidence that the plane of separation is along the original longitudinal split of the chromatin thread.

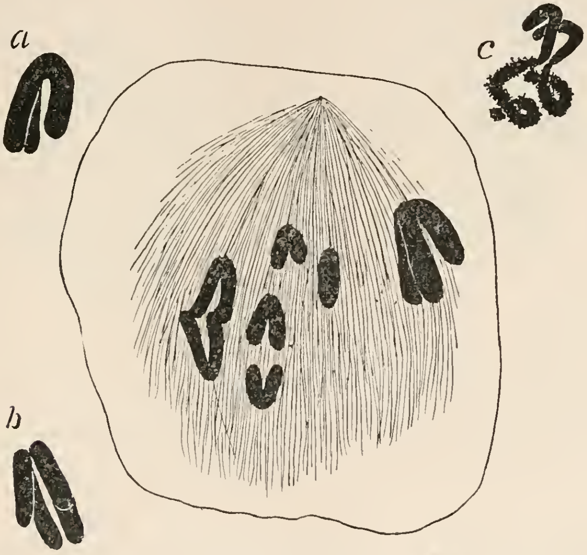

Fig. 13. Early anaphase of first spermatocyte in *Mermiria*. Only one pole of the spindle in the section and a few of the chromosomes. The method of the dyad separation shown in the tetrads. At the right of the spindle is represented a tetrad derived from the decad. Similar elements are shown in "*a*" and "*b*." At "*c*" is drawn the same structure in the telophase, where the homogeneous character of the accessory chromosome is in evidence.

A careful enough study of these cells has not yet been made to determine whether the pair of chromosomes in the second spermatocyte that remain united is the one which reappears in the spermatogonia of the next generation or not. It may not be possible to ascertain this positively under any circumstances, but I believe that definite enough information could be secured by a comprehensive study. Considerable importance attaches to this as will be shown later.

In the Tryxalines the organization of all the cell contents is complete and precise at the time of the first spermatocyte mitosis. Practically the entire cell takes part in forming the bipolar mitotic figure. It is interesting to observe that the greater portion of the cytoplasmic fibers lie outside of the circle of chromosomes that show in a polar view of the metaphase. At this time the chromosomes are scattered along in the spindle, forming no definite plate, and seem to divide independently and unaided.

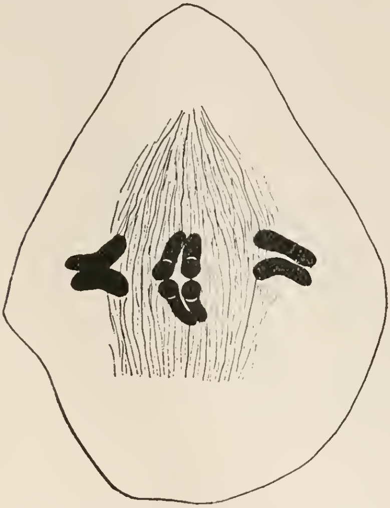

FIG. 14. Portion of a second spermatocyte of *Mermiria* in which the division of the tetrad coming from the decad of the first spermatocyte is shown along with that of the dyads.

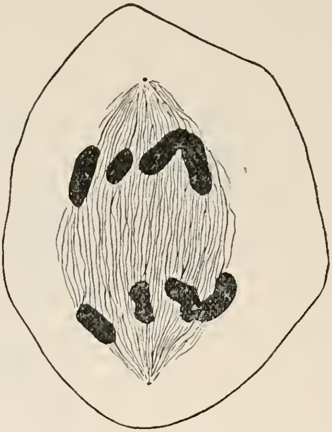

FIG. 15. Anaphase of the second spermatocyte of *Mermiria* showing the separated dyads of the tetrad and halves of ordinary dyads.

This independent action of the chromosomes and the disproportion between them and the cytoplasmic figure would indicate apparently that the formation of the spindle is not to produce a mechanism for the separation of the chromosomes, but is rather for the purpose of securing an accurate division of the cell materials. This assumption is further supported by the observation that the same chromosomes that are involved in the first spermatocyte mitosis, where the spindle fills the entire cell, in the preceding generation, the spermatogonia, divide around a spindle that is so small as to be practically negligible as a motor apparatus for the movement of the chromatin. The spermatogonial spindle is indeed so diminutive as to be almost invisible among the relatively huge chromosomes that surround it, and so short as to be a fraction of the length of the chromosomes.

If the mitotic figure is purely, or even principally, a mechanical arrangement for the separation of the chromosome halves, then there would be some proportion between the spindles of successive generations which have to do with the same chromosomes. The ability of the chromosomes to execute independent movements is proven by the action of the accesosry in wandering to one pole of the spindle in advance of the other elements, as well as by the long series of movements performed by them in the prophases.

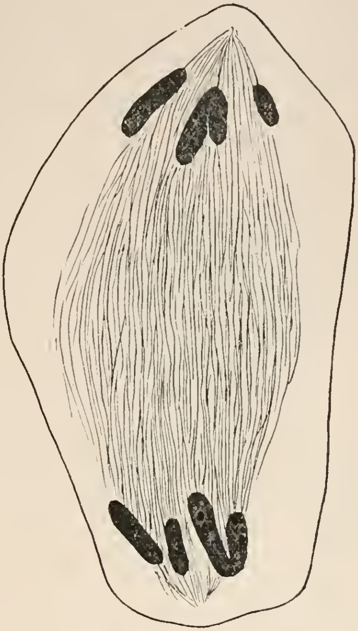

FIG. 16. Late anaphase of second spermatocyte of *Mermiria*. Here are represented later stages in the separation of such elements as are shown in the preceding figure.

4. *Certain Chromosomes of Chortophaga viridifasciata.*

Besides the instances of unusual chromosome associations mentioned for *Hesperotettix* and *Mermiria* I am able to bring forward others which will serve to show that the conditions present in these forms are not pathological or abnormal. The most pronounced case of synapsis of spermatogonial chromosomes is exhibited by *Chortophaga*, an Œdipodinæ. I observed and photographed cases of this three years ago, but did not attach much importance to the condition until I recently encountered it in a number of other forms.

The spermatogonia of this genus show numerous pairs of chromosomes when viewed from the pole, in which the evidence of the end to end union of univalent chromosomes is unmistakable. The number of these pairs is not constant, but as many as four have been encountered in one cell. A side view of an anaphase shows very clear heterotypical divisions among the chromosomes, the remainder of which are divided in the usual way. The subsequent history of these I have not worked out.

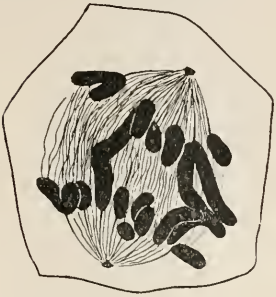

FIG. 17. Lateral view of a spermatogonium of *Chortophaga* in metaphase. The separation of multiple chromosomes along with simple ones is well shown. This is another good illustration of heterotypical divisions in the spermatogonia.

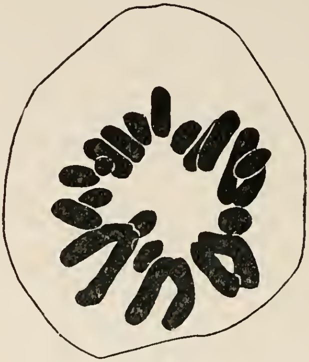

FIG. 18. A polar view of a similar cell in which appear four tetrads. This is an exceptionally good illustration of early synapsis of the chromosomes.

5. *Certain Chromosomes of Anabrus.*

During the course of my studies upon Locustid genera I was caused not a little difficulty by an oddly formed chromosome in the spermatocytes of the genus *Anabrus.* At the time I was obliged to confess myself beaten in the endeavor to determine the constitution of this element. That it had to do with the accessory chromosome I was thoroughly convinced, but that it was unusual in some way I was equally assured. Not until I had become familiar with the hexad multiple chromosome of *Hesperotettix* did the matter clear itself up. Having learned the possibility of an association of the accessory chromosome with one of the bivalent structures of the first spermatocyte I was soon able to see that the peculiar chromosome of *Anabrus* was another such a multiple chromosome as that of *Hesperotettix*. There is this difference between them, however, that in *Hesperotettix* the accessory chromosome is the small, univalent element, while in *Anabrus* it is proportionately very large, causing the tetrad with which it is associated to appear as an appendage at one end.

The multiple chromosome of *Anabrus* is a striking illustration of a fact to which I have often made reference, *i. e.*, to the relation existing between chromosomes and the attached archoplasmic fibers. *In all tetrad elements the fibers are annectent at the fused ends of the chromosomes, representing the plane of the future reduction division, and never at the free ends.* When the accessory is added to a tetrad in *Hesperotettix* the arrangement is disturbed somewhat in order to allow the insertion of one fiber to the point of contact between the accessory chromosome and one member of the tetrad, while the fiber to the other centrosome finds its connection at the free end of the tetrad.

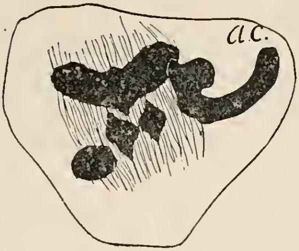

Fig. 19. Oblique view first spermatocyte of *Anabrus* in metaphase in which appears a multiple chromosome consisting of the accessory chromosome "*a.c.*" and a small tetrad, several small tetrads and the one large one.

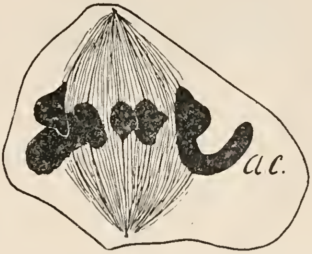

Fig. 20. Lateral view of first spermatocyte of *Anabrus* in metaphase exhibiting the same structures represented in the preceding figure. Note the attachment of the fibers to the hexad.

Such a condition obtains also in *Anabrus* resulting in the production of a very singular appearing chromosome. Since the accessory is so very long it seems as if the fibers from both centrosomes attach near one end of the multiple chromosome, but as a matter of fact one is connected where the accessory joins the tetrad and the other at the opposite end of the tetrad. From the variation in shape of the tetrad portion of the hexad it would appear possible that it has the accessory attached to the side and so divides longitudinally, but this I have not yet determined. Frequently it happens that the free extremity of the long accessory chromosome lies near the pole of the spindle, but in metakinesis it is the other end that moves toward the centrosome. In its passage from the equatorial plate to the pole this end of the chromosome is bent upon the other moiety producing a U-shaped figure. I have not had opportunity to trace the later history of chromosomes thus associated, but have little doubt that they conduct themselves after the manner of similar ones in *Hesperotettix*.

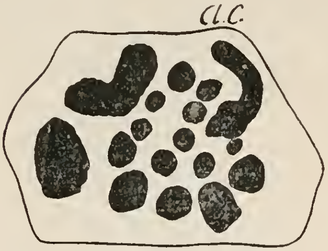

FIG. 21. Polar view of a similar cell with all the chromosomes drawn.

It is thus shown that in three of the Orthopteran families, *i. e.* Acrididæ, Locustidæ, and Phasmidæ (de Sinéty) a hexad multiple chromosome is found in the first spermatocyte of certain species. Doubtless it exists in many others not yet studied and may reasonably be expected to occur in all the families of the order. In the presence of such elements we may also find an explanation of differences in number and behavior of chromosomes in related forms and in such as exhibit peculiar chromosomes not referable to the ordinary types.

SUMMARY OF OBSERVATIONS.

1. An Orthopteran family, the Acrididæ, is characterized by the possession of a definite and fixed number of chromosomes in its germ cells.

2. A genus of this family is marked by the arrangement of this series of chromosomes in a characteristic manner, which results in a precise sequence of divisions for these elements.

3. A species shows the grouping of chromosomes peculiar to the genus, but is distinguished by size differences of chromosomes, spindles and other cell parts.

4. The synapsis of chromosomes in pairs or other combinations does not occur in all species at the same time, nor do all the elements of the cell enter into these relations synchronously. There is accordingly no fixed synaptic phase.

5. Heterotypical divisions are observed in spermatogonia, first spermatocytes, and second spermatocytes, and in each case represent an ordinary longitudinal cleavage of the chromatin thread.

6. In cases of unusual associations of chromosomes the accessory is involved in the formation of the multiple chromosome.

7. Under all conditions the accessory chromosome goes to but one half of the spermatozoa.

8. When pentivalent multiple chromosomes are formed whole tetrads go into the second spermatocytes undivided.

10. In a single mitosis some of the chromosomes may divide qualitatively while the others divide quantitatively. This is due to unusual associations of the elements, and occurs regularly in the same way in all the cells of a generation.

11. Unless chromosomes are associated into multiple elements of higher valence than tetrads they divide longitudinally in the first spermatocyte and transversely in the second.

12. Tetrads divide longitudinally wherever found, either in spermatogonia, first spermatocytes or second spermatocytes. Multiple chromosomes of higher valence than tetrads divide transversely.

Conclusions.

1. *Chromosomes and Somatic Characters.*

Two facts of first importance stand out as a result of these observations. The first is that a definite series of chromosomes accompanies the exhibition of a group of somatic characters that have been utilized by systematists for the inclusion of a number of genera into a family. The second fact is that these genera are characterized by a very precise architecture of their germ cells, whereby the series of chromosomes presents a definite arrangement and association which suffers modification in minor details in the different species of the genus. At the bottom of all, of course, lies the general fact that the chromosomes are morphological elements of the greatest constancy and importance.

Much has been done lately to show that the theory of the individuality of the chromosomes is well founded, but up to this time all that has been attempted has been to prove that in the species there is a constant series of chromosomes that appears in the successive generation of cells in each individual. I think that this has been done beyond reasonable question, in a number of forms and by different means. From the brief comparative study of the Acrididæ that I present here it now appears that not only are the chromosomes constant for the species, but also for the genus and family.

The far-reaching importance of this correlation between somatic characters and germ cell structure is at once apparent, for there is now some hope that it will be possible to establish the relations that exist between body characters and individual chromosomes. This has been the goal toward which I have been working ever since I observed the constancy in the behavior of the accessory chromosome and surmised its connection with the development of the male sexual characters — the first attempt, so far as I know to establish a relation between a particular chromosome and the same group of characters in different species.

The problem is unquestionably one of great complexity and difficulty, but I believe that with careful comparative studies of a great number of genera the rôle of the individual chromosome in development can be ascertained. If it is found possible, as I hope it may be, to apply experimental methods in breeding, etc., to these forms the results may be more quickly brought about. I have already made some tentative experiments, but have as yet accomplished nothing definite. Increased knowledge of the phenomena prevailing in these animals is bound to make the correlation between germ cell architecture and body characters more and more definite in details, and in connection with observations on other forms, will permit the formulation of some general principles that will be applicable to all organisms.

In the present state of our knowledge, however, we can do little more than erect a working hypothesis of a very general character, which doubtless will have to be modified with the apprehension of new groups of facts. It is to be borne in mind that our hypothesis must explain not only the appearance of the characters in the individual and groups of individuals, but must also offer some explanation of the possible origin of new characters which serve as the basis for new genera and species — or, in other words, of variation. The most we can hope to accomplish by observation, in any event, is to determine the mechanism by which the germ cells operate to produce the body structures — the principle back of this can only be guessed at.

Before undertaking the formulation of any hypothesis it may be well to consider a few questions concerning the nature of body characters and their relation to each other and to the germ cells. The term "character" has a very broad and uncertain meaning. It may be applied to any structure of the organism from a vitally important organ to a trivial marking on the surface of the body. It is very much a question whether the characters belonging to these categories are equally firmly fixed upon the germ plasm, and for our present purpose it is desirable that we know whether there is any relation between the need of a character and its fixity in the chromosome. Again we should know the relations of the characters to each other in development. We shall have to ascertain, for instance, whether an organ or part develops purely as a result of the presence of one particular chromosome or whether the structure owes its initial stages to the action of one chromosome and its later development to the influence of another. It will be necessary to determine to what extent the chromosome acts as an individual and how far it functions as a mutually coöperating element in a complex. These considerations lead finally to the question as to the nature of the individual chromosome at different periods in the development of an organism. Is the chromosome of the just mature germ cell potentially the same as that of the tissue cell in the adult organism, or has the latter become progressively different throughout the ontogeny of the organism? If the second condition obtains then it is necessary to determine the means by which the chromosomes of the germ cells maintain their primitive nature through successive generations while the somatic cells in each acquire their various characteristics.

These considerations force themselves upon one as soon as the attempt is made to establish a relation of any sort between an individual chromosome and certain characters of the body. Unfortunately we have no answers for these questions and they become, in fact, a part of the general problem.

As has been stated, the ultimate aim of these studies is to determine the relation between individual chromosomes and characters in the body, but this specific knowledge will come last of all and only after the most extensive investigations, so it is needless to say that as yet nothing has been discovered concerning the suspected relationship. What will first be accomplished will be the determination of the nature and behavior of the chromosomes in different species and from these observations some suggestions regarding the mechanism of the chromatin elements in heredity may be advanced. This knowledge may come about by a comparison of the germ cells and body characters in nearly related species, by observing the differences in germ cells of individuals that vary from the type of the species, or finally by experimentally disturbing the normal conditions in the germ cells and observing the effects upon the body. An attack on the problem must begin, then, by a search for differences between the germ cells of related species and between those of related genera, and in the present instance concerns certain forms that show very striking arrangements of the elements.

Upon the theory of the primary importance of the chromosomes in heredity we are forced to assume that the development of the characters in the individual of the genus *Hesperotettix* is the result of a definite composition and arrangement of the chromatic elements in the germ cells. Since definiteness of number, composition, and arrangement of the chromosomes always precedes the exhibition of a certain series of characters in the organism, it must be true that an alteration in either the number, composition, or arrangement of the chromosomes would be followed by the development of a somewhat different series of body characters. As I have previously mentioned, the number of chromosomes does not vary, nor is it possible to detect any difference in the composition of the chromosomes (although of course it occurs), but a characteristic arrangement of the chromosomes distinguishes this genus from others in the family, and so we must conclude that this is genetically connected with the subsequently appearing characters.

Within the well defined genus *Hesperotettix* occur the three clearly marked species, *pratensis*, *speciosus* and *viridis* and the germ cells of these exhibit the same grouping of chromosomes — at least of the ones most clearly distinguishable. Very probably when the complex is more thoroughly studied and understood there may be detected variations that are now obscure. Taking the case of the hexad multiple chromosome as being the clearest element to distinguish in the present state of our knowledge, it is found that in *viridis* (?) the tetrad portion shows a pronounced difference in size from that of the corresponding element in *speciosus*. Along with this are associated differences in the form and size of the spindle, etc. — a condition that may accompany, or be caused by, differences in the chromosomes. These peculiarities are connected with the exhibition of the body characters that are utilized for the purpose of classification. Whether these particular structural peculiarities are of the first importance or otherwise we have no way of determining, and this may delay the progress of our knowledge if there be differences in the power of transmission between characters of different rank in functional importance. We can not, therefore, with our present information, say, for example, that the variations of the structures mentioned are the cause of the difference in the size and proportion of the body in the two species, or whether they have to do with the surface markings on the exoskeleton. But when we know the full history of each chromosome in the complex and can follow its variations in all the species of the genus and can know how it differs from others in nearly related genera, then we may be able to associate a particular chromosome with a definite group of characters.

However, in these minutiæ we shall have to await further and much broader knowledge, and for the present turn our attention to the more general questions that have centered around the larger cytological phenomena in their relation to body characters. It is not my intention to take up the question of the form and sequence of the maturation mitoses, since I intend to treat these matters *in extenso* in a later paper. It will suffice to say here that the enlarged views gained by more recent study have tended only to confirm me in the opinions that I have previously expressed regarding these matters. Just now I wish to consider the bearing that the peculiar multiple chromosomes of *Hesperotettix*, *Mermiria*, *Chortophaga* and *Anabrus* have upon the general questions of heredity raised by the experimental work of the Mendelian school and by de Vries and his followers.

2. *The Chromosomes and Mendel's Laws.*

The first attempt to establish a correlation between cytological phenomena and the operation of Mendel's laws was made by Sutton in 1903, and was based upon Orthopteran material prepared and studied to a large extent in my laboratory. It was in thorough concordance with the laboratory results and represented the final step in the establishment of the theory of the individuality of the chromosomes and their relation to body characters. Done independently of Montgomery, it yet had as its fundamental idea the belief that half the chromosomes are paternal and half maternal, and that at the time of numerical reduction there is a union of homologous chromosomes in pairs. Montgomery clearly stated this idea of the union of parental chromosomes and should have due credit for it, but it is to Sutton's work that we are indebted for the detailed evidence of this fact, and for its theoretical application to the appearance of alternative characters in normal and hybrid breedings. It is this idea of definite association between chromosomes and characters and the explanation it offers for the purity of the germ cells and the recession of traits that renders the work of Sutton unique and makes it valuable.

There is no necessity for entering into any extended discussion of Sutton's results because, coming opportunely as they did with the renaissance of Mendel's conceptions, they immediately gained attention and are generally well understood. The essential feature of his conclusions is that there are in the somatic and immature germ cells a double series of chromosomes, one derived from the father and one from the mother. In synapsis this double series is united into one by the pairwise fusion of homologous chromosomes which remain in this state until the reduction division when they are separated again into a series which is of neither purely maternal nor paternal origin. This is offered as an explanation of the purity of the germ cells postulated by Mendel.

Sutton's views are formulated from careful comparison of size relations of chromosomes in the different generations of the male germ cells of *Brachystola* and represent the detailed proof of the conception expressed in more general terms by Montgomery.

Regarding the main view that the reduced number of chromosomes in the maturing germ cells is brought about by definite fusion of simple chromosomes into multiple ones of bivalent value, there now appears to be a pretty general agreement, and the older conception of a variety of processes has given way to the conviction that the same plan may be detected in all organisms when sufficiently studied. The final step in this direction is to regard this fusion of elements as a definite one between the same members in different generations of organisms. Such a correlation between germ cell architecture and somatic structure would be purely theoretical were it not for the fact that in the operation of Mendel's law there is an exact parallel between what is accomplished for the chromosomes in the maturation divisions and for particular characters in hybrid matings. While this is not an exact proof it raises the probability almost to a certainty and makes it practically impossible to doubt the accuracy of the hypothesis so far as it applies to purely Mendelian characters.

It is known, however, that many characters do not follow the course of those denominated Mendelian, and it now appears from the observations recorded in this paper that the simplicity of behavior by the chromosomes, assumed always to occur at the time of germ cell maturation, is not universal. The question naturally arises, then, whether the extremely simple explanation offered by Sutton is sufficiently inclusive.

Attention has already been called in previous papers to the probable importance of the prophase of the first spermatocyte in the matter of the hereditary transmission of characters. Upon the theory of the union of homologous chromosomes into pairs by synapsis[1] the nature of this becomes evident, for in the grasshopper this pairwise union of chromosomes continues in existence through fully half the life of the individual. If the chromosomes are centers of specific energies it is at this time that they would best be able to influence each other. Montgomery regards this as a phenomenon parallel to what occurs in the conjugation of the lower organisms, and so considers it a process of rejuvenation. This is a very loose term, and according to Montgomery's conception seems to mean nothing more than a stimulus to growth, for it is his conclusion that because of the conjugation of the chromosomes the germ cells immediately proceed to increase in size and attain dimensions in excess of those reached at any other period of their existence.

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[1] By synapsis I mean the fusion of simple chromosomes into multiple ones, usually of bivalent value, according to the idea of Moore, who proposed the term. I would suggest that in order to avoid the lamentable confusion that has resulted from the misuse of this designation that a new descriptive word be applied to the condition of the nucleus in which the chromatin is found massed at one side of the vesicle, without regard to whether it is a normal phenomenon or not. To carry out this idea I shall call this stage the "*synizesis*" of the chromatin.

To me there is a much deeper meaning in this intimate and long continued association of the chromosomes which, as I have recorded for *Chortophaga*, may be established in the early spermatogonia. If there is to be a balancing of forces between homologous chromosomes this would seem to be the place where it must occur. We find, as a matter of fact, that in normal breedings there are produced germ cells that are either entirely pure in regard to certain characters or that develop intermediate results between the two extremes of the parents. The same individuals may show the alternative inheritance of one character and the blended inheritance of another. These phenomena would seem susceptible of a reasonable explanation if we should consider that in the first instance the interaction between the homologous chromosomes was slight, or entirely lacking, while in the second case it was more extensive. Upon the occurrence of the reducing division the linked chromosomes of the first example would separate practically unchanged, while in the other they would pass into the germ cells with different potentialities than they possessed before their synapsis.

If parents of different species were employed we might expect the same results and secure second generations of inbred hybrids that would show pure parental characters or various blends of these. Mosaics might easily be accounted for if we assume that the exchange between the chromosomes has been of such a character as to produce nearly a balance of the alternative characters. Through inequalities of division or by reason of different environments, now one character would appear, now the other, and so produce the piebald result. Again we might conceive that the difference between two alternatives was so considerable and the disturbance of normal conditions so violent that the whole organization of the chromosomes would be upset with the result that a character distinct from that of either parent would be produced. These various alternatives are realized experimentally and I can see no simpler or more consistant explanation for them than the one I have presented.

3. *Multiple Chromosomes and the Chromosome Complex.*

As I have indicated already, there occur in *Hesperotettix* and *Mermiria* peculiar associations of chromosomes that are consistent from generation to generation of organisms. This can be no accident and undoubtedly has some fundamental meaning. It seems evident that the characters owe their orderly sequence in development to the organization of the germ cell elements and not alone to the mere presence of a particular chromosome. The experimental work of Boveri upon echinoderms and *Ascaris* would indicate the high importance of the individual chromosome, while the observations of Conklin upon the ascidian egg argues strongly for the view that what the chromosomes are able to accomplish in development depends largely upon the materials on which they have to operate — although we must not forget that these materials are probably produced by the action of chromosomes that came from the same original cell as do those of the germ cells.

It seems to me that these apparently somewhat contradictory observations speak unmistakably for the idea of precise organization in the germ cells and for mutual interaction between their parts. This thought comes home to me with particular emphasis after a study of the unusual precision in the arrangement of the cell parts of the spermatocytes of *Mermiria.* The evidence of the pentivalent multiple chromosome is of special importance for it shows that in order to maintain the identity of the chromatin complex from generation to generation there must be an accuracy not only in the division of the chromosomes in maturation but also in their coming together in fertilization. If it were not for this definiteness of separation and recombination of the chromosomes then there would be all conceivable combinations instead of a definite and constant one such as is always found. An examination of the conditions in this genus cannot fail to put us in possession of some facts that could not well be obtained in any other way. It is necessary to go back to the early first spermatocyte prophase to secure an understanding of the structure of the chromosomes — a point that I have always insisted upon since the beginning of my work upon spermatogenesis. Here it is found that one of the tetrads has become united end to end with the accessory chromosome so that the planes of their longitudinal division are coincident. As is invariably the case in Orthopteran cells the accessory chromosome is univalent, so that there is thus produced a trivalent chromosome like that of *Hesperotettix*. Throughout the length of the chromosome there is a clear longitudinal split which, along the portion contributed by the accessory chromosome, is sharply marked because of the homogeneous character of its elements, while in the remainder of its length it appears somewhat interrupted on account of the granular structure of the tetrad region.

Some time near the dissolution of the nuclear membrane there is joined to this trivalent chromosome, by end to end union, one of the tetrads, thus producing a pentivalent element or decad. This element, we know, has a division running its length and corresponding to the longitudinal split of the chromatic thread, although in its homogeneous condition it is, like that of all the other chromosomes, obscured. In the metaphase of the first spermatocyte, as I have before stated, the separation of the chromosomes takes place in such a way that to one of the daughter cells there goes a tetrad and to the other a tetrad plus the accessory chromosome. For these elements the first maturation mitosis represents a reduction division and one of a very unusual character. At the same time, however, the other chromosomes are dividing longitudinally. Of this there can be no doubt for in the Tryxalines the ring chromosomes are numerous and in *Mermiria* as many as five or six may be found in one equatorial plate. This evidence is unmistakable to one who has a knowledge of their structure gained from a study of their formation in the prophase, and their position in the equatorial plate, together with their relation to the mantle fibers in the metaphase and anaphase. I cannot stop here to enter into a criticism of the entirely erroneous interpretations of these rings by which de Sinéty proves a double longitudinal division and Montgomery a prereduction.

The probability of the occurrence of equational and reduction divisions in one mitosis is one against which I have previously argued strongly, because whenever reported the cases have lacked the support of thorough observation. The failure of the accessory chromosome to divide in the first spermatocyte mitosis, which is equivalent to a reduction, was the first authentic instance of this complexity, and now we have the case of the multiple chromosome in *Mermiria*, which furnishes further indisputable evidence of the two types of division in one mitosis. In the face of this evidence we must accept the fact and seek an explanation.

This is a matter of secondary importance compared with the question of the probable distribution of the chromosomes to the four spermatozoa arising from each first spermatocyte. It would appear at first glance that from the complexity introduced by the unusual arrangement and distribution of the chromosomes in *Mermiria* there must be a great many possibilities for variation in the chromosome complex, but if we accept the theory of the continuity of the chromosomes and remember that always the same complex appears in each first spermatocyte, then we must conclude that the mechanism of separation, distribution and recombination is a precise one. On the other hand, it is evident that the spermatozoa cannot all be alike, and we have to deal with different categories. This fact has been recognized for some time with regard to the accessory chromosome, but by the unusual combination of parts in the multiple chromosome (decad) of *Mermiria* there comes about an additional differentiation. The precise nature of this I have not been able yet to determine, on account of the absence of some prophase stages in my material. This hiatus I hope to remedy by the acquisition of a larger series of slides, but meanwhile we are sure of some facts and these are sufficient for the present discussion.

Since entire tetrads pass into the second spermatocyte there are produced four sorts of spermatozoa with regard to the combinations of chromosomes, but two kinds with respect to the presence or absence of certain ones of a multiple. In order that always the same combination of chromosomes (so far as size is concerned, and this appears necessary to satisfy the observations) should appear in the spermatocytes, it would be necessary for eggs with the missing elements to be present and to be fertilized with the proper spermatozoa. The exact nature of this coördinate action cannot now be determined for we are lacking sufficient data, but that it must exist seems apparent from the observation that always the same sized complex reappears in each generation of first spermatocytes. From even our limited knowledge it seems evident that there is great precision and definiteness of organization necessary to bring about in successive generations of similar cells the same grouping of chromosomes. That the entire cell is involved in this organization is, I think, indicated by my observations on the spermatocytes of *Mermiria* where it appears that practically the whole of the cell material is bipolarized and thus accurately divided by mitosis.

The fact that there exists an orderly sequence of concerted movements on the part of the chromosomes suggests many interesting possibilities and offers opportunities for many speculations and theories, but it would seem wise to await the determination of more facts and a careful coöperative study of the species involved before venturing into speculation. Because of the importance attaching to these observations it has seemed desirable to make them known now in the hope that similar phenomena may be observed in other objects. Every additional fact of this sort largely increases the prospect of our attaining an understanding of the mechanism of the hereditary transmission of characters, and if it can be found that a peculiar association of chromosomes is always accompanied by a characteristic arrangement of the body parts then we may begin to see the relation between germ cell architecture and somatic structure.

Among the uncertainties in my mind concerning the behavior of the chromosomes in *Mermiria* is one relating to the nature of the association of the chromosomes into the multiple element of the first spermatocyte. The tetrads seem of the usual type, *i. e.*, have simple chromosomes of equal size, but when the decad divides it would appear as though there were some heterogeneity present, for in the anaphase one limb of the loop is longer than the other (Fig. 13). This may be due to the formation of a multiple chromosome partly from the accessory chromosome; otherwise it means that the tetrad is not constituted of homologous simple chromosomes. Aside from this there seems to be nothing to contradict the view that the tetrads represent the union of homologous paternal and maternal elements.

4. *Phylogeny of the Chromosomes.*

By the determination of the fact that for the family Acrididæ the number of chromosomes is a constant we gain a new viewpoint for the study of chromosome descent. So long as it was thought that nearly related species were characterized by the possession of different numbers of chromosomes it was impossible to regard the individual chromosomes as very constant structures. We have accordingly such theories as Paulmier's, later endorsed and elaborated by Montgomery, by which certain chromosomes smaller than the others and somewhat different in their behavior in the mitoses are regarded as disappearing from the species and carrying with them the loss of certain characters.

On *a priori* grounds such an occurrence would seem to be very improbable, for the difference between any two species is so slight that the loss of any number of the chromosomes would be entirely disproportionate to the effect. As a matter of fact the difference between two species or two genera consists not so much in the presence or absence of certain characters, but rather in the modification of those possessed in common. If therefore we have a constant relation between a certain group of chromosomes and a certain series of body characters we would expect differences between individuals to come about, not by the loss of chromosomes but by the modification of their structure or relations. It seems to me that the incomplete observations which I am presenting in this paper strongly indicate that this is what takes place.

The Acrididæ are a group of grasshoppers well marked off from the other Orthopteran families by the possession of a series of characters that are remarkably constant. It is, in fact, sometimes difficult to establish characters of sufficient importance to differentiate subfamilies by their presence or absence. This precision and fixity of bodily organization is accompanied by a corresponding stability of the chromosome complex, which indicates that throughout the family there is a correspondence between individual chromosomes and their products in development. By this I mean that if we can fix upon any particular chromosome by some peculiarity of structure or behavior and associate it with definite body characters we will find that same chromosome in all the species of the family governing the development of the same somatic structures. Individual chromosomes have therefore a continuity of descent in the same way that cells and organisms have.

Comparing the Acrididæ with the Locustidæ it appears that in the spermatogonia there is a difference of ten chromosomes in favor of the latter group. While these two families are well separated it is due more to differences of common characters than to the absence in one of characters possessed by the other. We must conclude, therefore, that the chromatin governing the development of these common characters is present in both families. It is evident, however, from the enumeration of the chromosomes, that it is differently associated in the two groups with the exception of that contained in the accessory chromosome. We cannot for this reason compare chromosomes with chromosomes throughout the whole complex of the two families and it is plain that the differences in structure are due to the differences in composition and association of the chromosomes. This is foreshadowed, or indicated, by the differences of association that prevail between genera, as is suggested by the appearance of multiple chromosomes. These associations may further point the way to an understanding of the differences in number between different families, the smaller one being brought about by the parmanent union of independent chromosomes, rather than by the entire loss of elements.

5. *Chromosomes in Variation.*

A discussion of the relation of chromosomes to one another in different groups leads to an enquiry into the relation of characters to each other in various individuals. As is well known, between parent and offspring there may be a difference of characters such that if a sufficient series of pairs is taken there may be found an almost continuous seriation. Such variation is appropriately called "continuous." On the other hand it occasionally happens that between parent and offspring there occurs a sudden and sharp rearrangement of body characters, which remains constant. As opposed to the continuous variation this is called "discontinuous." Biologists are more and more inclined to place the origin of variation between parent and offspring in the germ cells and more specifically in the chromatin. Since, however, the types of variation are sharp and distinct there must be corresponding differences in the chromatin. We know so little about the relation this nuclear material bears to the development of characters that it is somewhat hazardous to venture an explanation of the mechanism involved. It seems to me, notwithstanding this, that we may gain some slight insight into the processes by a comparison of the chromosome groups.

Thus in the genus *Hesperotettix* the chromosome complex seems to be a fixed one, and in the different species there occur peculiarities that are constant. We are therefore warranted in assuming that if this uniformity were disturbed it would result in the development of a different series of characters. If, for instance, the hexad of the first spermatocyte in *Hesperotettix* should, for some reason, not be formed at the usual time of the coming together of the tetrad and the accessory chromosome, thus producing a different distribution of the chromosomes into the four spermatocytes, I think we may fairly assume that these cells would not cause the development of the group of characters which we associate with the genus *Hesperotettix*. In such an event we would have a mutation. This, of course, does not explain *why* the mutation occurs, but it accounts for the means by which the result is accomplished. There is in force here, possibly, the same principle that paleontologists have observed to be in operation in groups of animals that are in course of extinction. Here it is noticed that strange and unusual forms appear, and the closing history of the group is marked by the breaking up of the heretofore constant type into numerous and heterogeneous subtypes. We do not know certainly that this is an occurrence of exactly the same nature as the present day observed mutation, but it is of the same character and is an evidence that stability of the germ plasm is disturbed by irreversible changes which, as I have pointed out, may find expression in different arrangements of the chromosomes.

Opposed to the sudden and pronounced changes in character combinations that mark instances of discontinuous variation stand the common examples of continuous variation, where there is present a graded series of inconstant and fluctuating variations. To my mind the two types of variation suggest different mechanisms of transmission. Discontinuous variation speaks of pronounced and irreversible alterations of the chromosome complex ; continuous variation, on the contrary, suggests the idea of small changes in individual chromosomes, due to the impossibility of mechanically producing two exactly equivalent daughter cells. These latter variations are minute and of every conceivable character, but are inconstant and indeterminate. As a basis for the occurrence of these fluctuating changes we have the observed fact of the lack of exact correspondence in the size of chromosomes and other cell parts in the various cells of an organism, and the probability of a difference in the interaction of the chromosomes in synapsis. I can not refrain from again calling attention to the strong evidence that identical twins offer in support of the theory that the characters are fixed by the composition of the germ cells. Since these twins are the product of a single egg it is evident that they must have exactly the same chromosome complex, which, in development, will bring about a very close parallel in the characters produced under their control. No other pair of germ cells from the same two parents will ever produce two individuals that bear the resemblance to each other that identical twins do, because, by the laws of chance, it is improbable that any two pass through synapsis and the maturation mitoses and emerge from them with their chromosomes constituted in exactly the same way. Ordinary twins, coming from different eggs, are no more alike than children of successive births although they are developed under practically identical conditions, thus removing the possibility of environmental differences. Therefore it appears that the fluctuating variations which occur in a series of organisms comprising a species are the results of minor differences in the chromosomes of the germ cells, due to the impossibility of exactly duplicating the conditions of union and separation of the paternal and maternal chromosomes in maturation.

Mutations on the other hand follow some unusual disturbances in the relations of chromosomes to each other, brought about by some change in the germ plasm whose cause we cannot now determine. So much, I believe, we may conclude from our present knowledge of the germ cells and their behavior in maturation and fertilization. As our detailed knowledge of the chromosomes increases we may be enabled to speak more definitely and certainly, but further information we must have and it is earnestly to be hoped that the chromosome groups of many species of these Orthoptera may be carefully and exhaustively studied in connection with observations upon body variation so that correlation between the two may be established.

Zoölogical Laboratory, University of Kansas,
June 21, 1905.

New Terms Employed.

Definitions and Classifications of Chromosomes.

Chromosomes are chromatin elements acting as unit structures during mitosis.

Chromosomes are of two general classes.

1. Simple — containing two chromatids in metaphase.
2. Multiple — containing more than two chromatids in metaphase and formed by the union of simple chromosomes.
    - (*a*) Tetrads, containing four chromatids.
    - (*b*) Hexads, containing six chromatids.
    - (*c*) Octads, containing eight chromatids (not yet observed).
    - (*d*) Decads, containing ten chromatids.

A chromatid is a half of a simple chromosome.

"Synizesis" — the unilateral or central contraction of the chromatin in the nucleus during the prophase of the first spermatocyte. A term proposed to avoid the misuse of the word "synapsis."

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BIOLOGICAL BULLETIN

THE DISTRIBUTION OF TRACHEÆ IN THE NYMPH OF PLATHEMIS LYDIA.

G. G. SCOTT.

The Odonata are regarded as among the most highly organized of insects. In fossil dragon flies found in the Tertiary are indications of high specialization. Dragon flies are especially adapted to swift flight. For this purpose they are supplied with broad wings and powerful muscles to work them. There is consequently a great need for a plentiful supply of oxygen. This is supplied to every tissue in all parts of the body by the tracheal system. On this account a study of the tracheal system is of interest and importance.

The late Professor James I. Peck, of Williams College, suggested to the writer in 1898 that he make a study of the distribution of tracheæ in the nymph of a dragon fly found near Williamstown, Mass. It is from notes and drawings made at that time that the present paper is written. The forms found were identified by Professor James Needham as *Plathemis trimaculata*, which name I am informed has since been changed to *Plathemis lydia*. Lubbock says that the larval tracheæ of insects are more generalized and represent more nearly the original type than those of the adult. The results of this investigation show clearly that on the other hand we have in the nymph of *Plathemis lydia* a very special and complex distribution of tracheæ in some respects especially adapted to aquatic larval life and changing to simpler conditions in the adult. Lubbock states that the distribution of the tracheæ depends on the size and shape of organs. Yet in the nymph of *Plathemis lydia* there are certain complexities that are not thus easily explained. For example the tracheæ of one side cross over and fuse with the tracheæ of the opposite

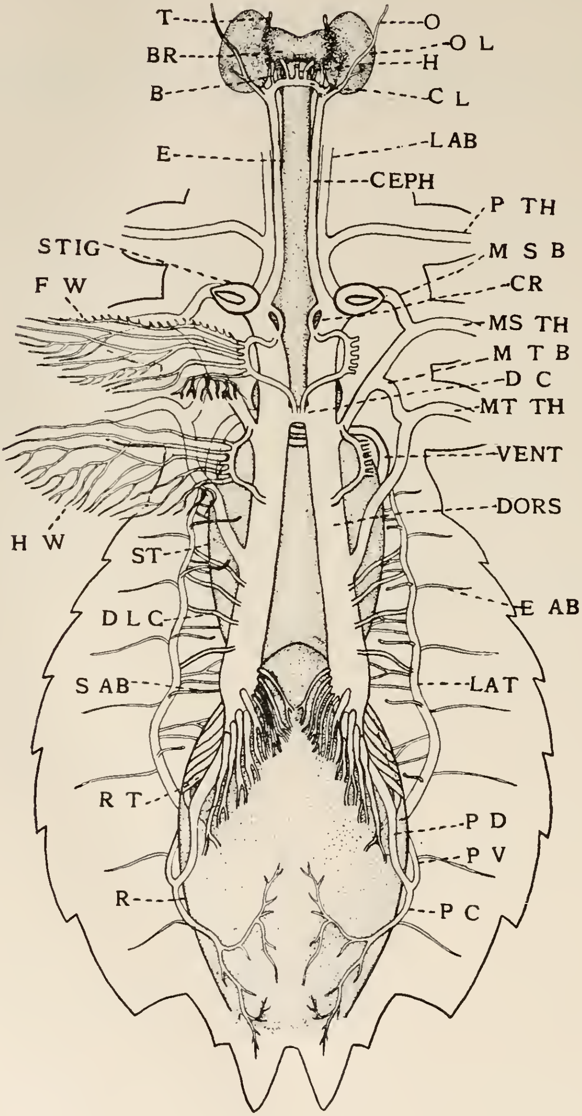
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FIG. 1.

side. The distribution in the main however follows the contour of organs or axis in parts.

Plathemis lydia belongs to the family Libellulidæ of the order

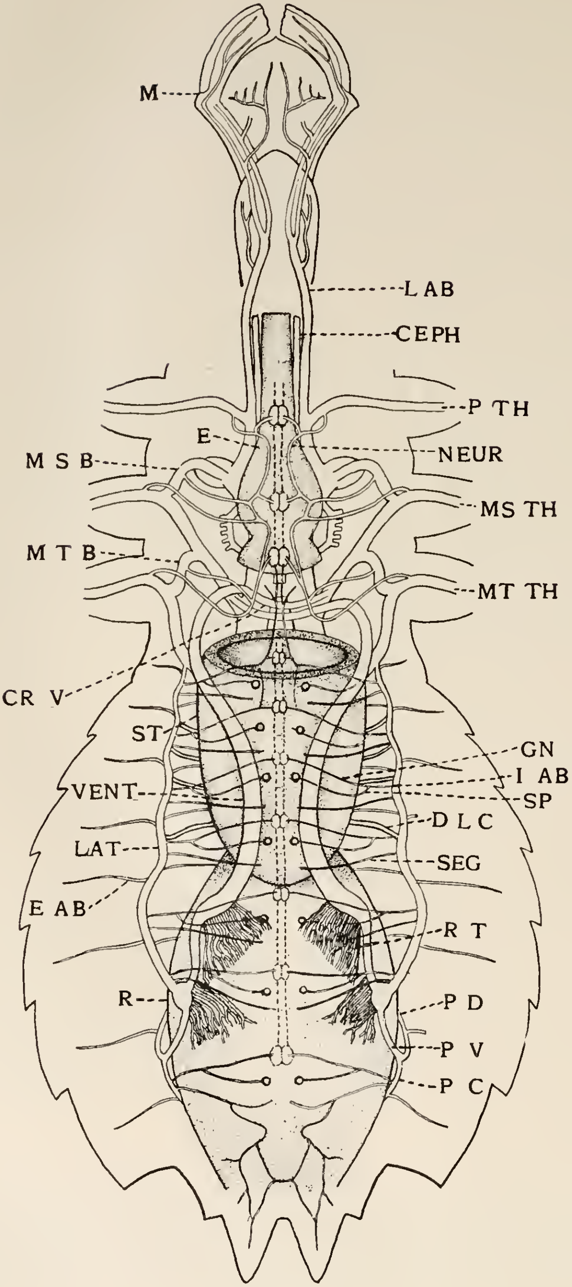
Odonata. The Libellulidæ are among the most highly organized of the Odonata. As far as the tracheal system is concerned *Plathemis lydia* is well classified. The nymph is aquatic, inhabiting the muddy bottoms of quiet pools. It has a swift darting motion when disturbed, caused by expelling water from the rectum. The average length of those studied was 23 mm., the width 7 mm. The head is marked by the large eyes at the upper outer angles ; a single pair of short antennæ extend from below and between the eyes. The prominent mask, a modified labium, covers the mouth parts. There are three pairs of legs, all attached to the thorax, which also bears dorsally two pairs of short functionless wings. Anterior to these is a single pair of prothoracic stigmata, functionless also. On the abdominal segments are found seven pairs of functionless stigmata. Internally the alimentary canal extends from mouth to anus and consists of esophagus (Fig. 1, *E*), stomach (Fig. 1, *ST*), intestine and rectum (Fig. 1, *R*). At the junction of the stomach with the intestine are the malpighian tubules. On the dorsal face of the gut over the rectum and stomach is the slightly lobed blood vessel. On either side of this and somewhat posteriorly are the immature reproductive organs.

Ventral to the gut is the chain of seven abdominal ganglia (Fig. 2) the posterior being the largest. The ganglia are connected by nerve cords to each other and by the same method anteriorly with the thoracic ganglia (Fig. 2). There are three of these not fused, the meta- and mesothoracic being nearer together than the meso- and prothoracic. The prothoracic ganglion connects by nerve cords with the subesophageal ganglion and this by the esophageal ring with the dorsally lying brain (Fig. 1, *br*). On the upper outer sides of the brain are the large optic lobes.

It is the tracheal distribution to the foregoing parts that I have worked out and also the connections and interconnections of the tracheæ.

METHODS.

The nymph was placed in a strong solution of glycerine in a watch glass and the dissections made with small scissors and needles sharpened down to cutting edges. Most of the work was done with a dissecting microscope. In the glycerine solu-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P V
P C

FIG. 2.

tion the tracheæ appeared as silvery white tubes. Some of the more minute points were worked out teasing with needles under the lower powers of the compound microscope. Glycerine mounts were easily made and studied.

Tracheal Distribution.

According to Packard '98 the tracheal system of *Plathemis lydia* belongs to the peripneustic type in which the prothoracic and abdominal stigmata are present though not functional during the greater part of the larval life. This type is intermediate between the holopneustic or open type and the apneustic or closed type.

The Dorsals. — The tracheal system is most conveniently understood by considering first those that lie dorsally, then those that are ventral and those that run laterally. In connection with each of these three systems there will be given an account of their principal branches. The connections of each of the others will be described. The stigmata found dorsally between the pro- and mesothorax will serve as a convenient starting point. From each of the stigmata (Fig. 1, *STIG*) the two largest tracheal tubes, the dorsal tracheæ (Fig. 1, *DORS*) extend posteriorly, diverging slightly to the sides of the rectum. There is a small crescent-shaped trachea (Fig. 1, *CR*) arising from the inner face of the dorsals at the stigmata and joining the dorsals again a short distance behind.

About one fifth of their distance back of the stigmata the dorsals are connected by a trachea of about one half their own diameter (Fig. 1, *D C*). The trachea to the mesothoracic leg (Fig. 1, *MS TH*) arises from the dorsal just back of the above mentioned connective. It receives a branch (Fig. 1, *M S B*) from the dorsal at the stigmata. The tracheæ to the metathoracic leg (Fig. 1, *MT TH*) arises from the dorsal trachea posterior to the origin of the mesothoracic. The metathoracic also receives a branch (Fig. 1, *M T B*) from the mesothoracic trachea. The function of these short branches is not clear. They may serve as stays to hold the meso- and metathoracic tracheæ in place. Small tubes pass from the thoracics to the vertical wing muscles.

To the Wings. — Just posterior to the hinder origin of the crescent arises the anterior limb of a loop (Fig. 1, *F W*) which furnishes the fore wing with tracheæ. The posterior limb of the loop passes back and joins the dorsal connective. The posterior limbs of both the fore wing loops join the dorsal connective side by side (Fig. 1). Five tracheæ pass out from the face of the wing loop. By the successive branching of these five tracheæ the whole wing is supplied. The first or the most anterior does not bifurcate simply but sends about twenty short branches to the edge of the wing. The second and third branches are the largest and supply the greater portion of the wing while the fourth and fifth send fine branches to the posterior margins of the wing. The distribution to the hind wings is similar to that of the fore wings. There are five main tracheæ originating also from a loop whose anterior limb (Fig. 1, *H W*) arises from the mesothoracic a short distance from its origin from the dorsal. The posterior limb of the loop joins the dorsal midway between the origin of the meso- and the metathoracic tracheæ. In the larva the legs are used much more than in the adult. The wings on the other hand are functionless in the larva. Therefore it is not surprising that the tracheæ to the legs are much larger in the larva. And yet although the wings are not used there is a complex distribution to every part.

Anterior to the Stigmata.— Two pairs of tracheæ originate from the dorsal at the stigmata, an inner or cephalic pair (Fig. 1, *CEPH*) and an outer or labial pair (Fig. 1, *LAB*).

The labials (Fig. 2, *LAB*) pass alongside the cephalics for a distance, then down around the esophagus and out to the mask (Fig. 2, *M*), sending out many branches. The distribution to the mask should be noted, as this organ is in constant activity in larval life for the capture of prey. The prothoracic trachea (Fig. 1, *P TH*) arises from the labial, a short distance anterior to the stigmata.

The cephalics (Fig. 1, *CEPH*) pass along the dorsal face of the esophagus and can be traced forward to the brain. There they are connected by a loop (Fig. 1, *C L*) and from this loop five branches pass forward. The middle branch (Fig. 1, *H*) passes dorsally to the top of the head. The two pairs on each side (Fig.

1, *B*) pass forward into the brain, indicating that a considerable amount of oxygen is used in the metabolism of this organ. At the place where the loop joins the cephalic tube two branches arise together — one the optic (Fig. 1, *O*) passing out to the optic lobes (Fig. 1, *O L*) and the eyes. The other to the trophic (Fig. 1, *T*) passes down underneath the optic lobes and on out to the head parts.

Posterior Dorsals.— The dorsals give rise to branches supplying the alimentary tract — the blood vessel and reproductive organs and other adjacent tissues.

Posteriorly the dorsals become much smaller in size, this being due to the fact that each gives off many smaller tracheæ to the dorsal and lateral surfaces of the rectum (Fig. 1, *RT*). Most of these after much subdivision into fine tracheoles pass through the wall of the rectum to the rectal gills. One of the larger branches of the dorsal continues back as the post-dorsal (Fig. 1, *P D*), then passes down over the side of the rectum joining the post-ventral (Fig. 1, *P V*), which curves forward and inward on the ventral surface of the rectum connecting with the main ventral tracheal tubes (Fig. 2, *VENT*). At the place where the post-dorsal meets the post-ventral the former continues posteriorly up over the dorsal surface of the rectum (Fig. 1, *P C*).

The Ventrals. — Arising from the post-ventral the ventral tracheæ (Fig. 2, *VENT*) pass forward on the ventral surface ot the rectum toward the mid line and diverge as they continue on the under surface of the stomach. At the anterior end of the stomach each ventral curves up and around crossing over the upper surface of the stomach. The right ventral finally joins the left mesothoracic trachea just beyond the latter's origin from the dorsal. Also the left ventral joins the right mesothoracic in a similar manner (Fig. *CR V*). Fig. 2 is drawn with the anterior part of the stomach removed to show the union of the ventral with the mesothoracics. It will be noted that the ventrals arising from the dorsals posteriorly join them again anteriorly. From the posterior ends of the ventrals tracheæ run to the ventral surface of the rectum and through to the rectal gills. In front where the ventrals are near together they send branches to the malpighian tubules and furnish the greater tracheal supply to the stomach.

Lateral System. — At the posterior origin of the ventrals there arises also the lateral tracheæ (Figs. 1 and 2, *LAT*). These run anteriorly lateral to the alimentary tract and near the outer lateral edges of the abdominal walls. The laterals run forward decreasing in size and join the metathoracics just beyond their origin from the dorsals. From the lateral trachea a branch runs out externally (Figs. 1 and 2, *E AB*) to each of the first six abdominal segments supplying the muscles which assist in the compression of the abdomen. Adjacent to these tracheæ others arise from the upper face of the lateral and run to the dorsal part of each segment (Fig. 1, *S AB*). Then a third set of tracheals originate from the laterals along with the others. These the segmentals (Fig. 2, *seg*) pass ventrally along the hinder margin of each segment sending branches forwards the width of the segment. Finally a fourth set of tracheal tubes arise from the laterals in each segment, the inferior abdominal (Fig. 2, *I AB*) so named because they pass below the alimentary tract. They bifurcate a short distance from their origin from the laterals. The anterior branch (Fig. 2, *GN*) supplies the near half of the abdominal ganglion of that segment. The posterior branch (Fig. 2, *SP*) runs out to the abdominal spiracle of that segment. In the nymph as before described these spiracles are closed yet the tracheal distribution to them is clear.

The first of the ganglionic tracheoles sends a branch forwards to the metathoracic ganglion and a branch back to the second ganglionic trachea. The seventh abdominal ganglion and spiracle has a different supply receiving a branch from the posterior continuation of the dorsal (Fig. 2, *P C*). Near this arises another trachea which with a similar one from the other side forms a network in the posterior and ventral part of the body cavity. Only the main features of it are shown in Fig. 2.

The distribution to the thoracic ganglia is more complex. Each of the thoracic ganglia receives on either side a branch from the neural trachea (Fig. 2, *NEUR*). This trachea takes a somewhat tortuous course the length of the thorax alongside the thoracic ganglia. It receives a branch from the prothoracic trachea—then just back of the mesothoracic ganglion it receives two branches, one from the mesothoracic branch (Fig. 2, *M S B*) and the other from the mesothoracic trachea itself (Fig. 2, *MS TH*). Finally the neural trachea receives two branches posteriorly. The first arises from the metathoracic branch (Fig. 2, *M T B*) the second from the metathoracic trachea (Fig. 2, *MT TH*). In addition the metathoracic ganglion as previously described receives fine tubes from the tracheæ which supply the first abdominal ganglion.

From the dorsal trachea posterior to the metathoracic there arise four tubes connecting the dorsal with the lateral trachea (Fig. 1, *D L C*). The lateral tracheæ at the anterior end are not much larger than these dorsolateral connectives.

Rectal branchiæ. — Like many of the larval dragon flies *Plathemis lydia* takes in air from the water which passes in and out of the rectum. The rectum of the nymph is proportionately much larger than in the adult. It is supplied with a complicated apparatus for separating the air from the water. The posterior end of the abdomen at the anus is provided with anal spines. There are three larger spines and a pair of smaller ones. Of the larger ones, two are ventral, one is dorsal while there is a single smaller spine between the dorsal and each of the ventrals. Just internal to these spines are three valves each adjacent to one of the larger rectal spines. The dorsal valve (Fig. 3, *D R V*) is the largest, the two ventral valves (Fig. 3, *V R V*) are of equal size.. Each edge of the dorsal valve is met by the external edges of the other two, a triradiate slit being thus formed when the valves are closed. The ventral valves seem to work on the dorsal valve as a base. When the valves close water is retained in the rectum. It is forced out again by the compression of the abdomen. If it is ejected with sufficient force the animal is propelled forward in the water.

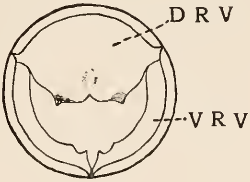

FIG. 3.

This compression of the abdomen is brought about by means of muscles connecting the roof and floor of the abdomen at its external edges. Expansion of the abdomen is due to two causes, first the relaxation of the above mentioned abdominal muscles and second the tendency of the walls to resume their somewhat cylindrical form. Extending the length of the surface of the rectum are six longitudinal bands or muscles (Fig. 4, *RM*) a dorsal pair, a lateral pair and a ventral pair. These muscles are equally distant from each other. To each of them internally is attached two rows of leaflike structures, the rectal gills (Fig. 4, *R G*) which extend into the cavity of the rectum. The dorsal trachea (Fig. 4, *D*) sends branches to the dorsal and lateral rectal gills. From the ventral trachea (Fig. 4, *V*) branches pass to the ventral rectal gills. Fig. 5 shows the relation of the gills (Fig. 5, *R G*) to the longitudinal muscles (Fig. 5, *L M*). In this figure the gills are pressed out laterally. The shortest edge of each gill is attached diagonally to the muscle so that the lines of attachment of the two rows of gills of the same muscle have the appearance of a succession of "V's," the diverging ends being toward the posterior. Each gill is somewhat triangular in shape (Fig. 6, *R G*) the proximal edge attached to the longitudinal rectal muscles being much shorter. At one corner of this shorter side is a somewhat triangular space (Fig. 6, *TR SP*) differentiated from the rest in general appearance. Sadones '95 describes it in the gills of other species and gives no decisive clue as to the function of it. He ascribes, however, an excretory function to it.

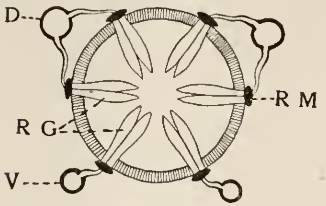

Fig. 4.

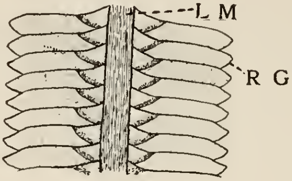

Fig. 5.

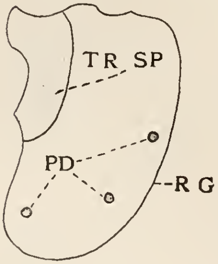

Fig. 6.

On one surface of the gill are three small pads (Fig. 6, *PD*) by which it is separated from the adjacent gills. The pads allow a thin film of water to form between each gill. Each gill is about a millimeter in length. Oustelet, '69, estimates that there are twenty-four thousand respiratory folds in the larva of *Æschna cyanea.* These folds are not flat enough to be called gills; they are more like papillæ, but they serve the same function as gills. There are certainly as many in the rectum of *Plathemis lydia.* By the great number of folds or gills the surface area of the rectal breathing apparatus exposed to the water is increased roughly speaking about eight or ten times. As previously indicated the dorsal trachea after many subdivisions divides into two sets of tracheæ (Figs. 1 and 2, *R T*), a dorsal set and a lateral set (Fig. 4, *D*), supplying the dorsal and lateral rectal gills. The fine subdivisions of the ventrals pass to the ventral rectal gills. The final disposition of the finer tracheoles to the gill itself is shown in Fig. 7. In this figure four gills are shown teased apart. Each tracheole (Fig. 7, *G T*) bifurcates just before it reaches the upper edges of two adjacent gills of the same side. One branch (Fig. 7, *P*) passes to the posterior edge of one of the gills, while the other branch (Fig. 7, *A*) passes to the anterior edge of the gill behind. Each of these branches sends finer tracheoles into the gill. These divide and fill the gill in its most expanded portion with a network of tracheæ.

Examination under high power shows that the tracheoles (Fig. 8, *T*) from the posterior and anterior gill tracheæ form loops overlying one another (Fig. 8, *AA*). The condition in *Plathemis lydia* then confirms the work of Sadones, '95, who found in the Odonata he studied that the gill tracheoles were found in loops and therefore connected. A close examination of his figures, however, shows that he has drawn the tracheæ ending blindly and not in loops.

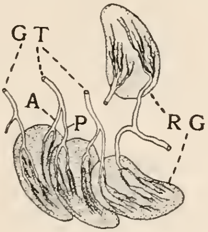

FIG. 7.

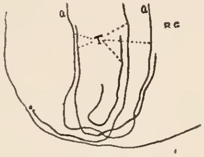

FIG. 8.

Oustelet and Palmén state that this rectal breathing apparatus does not disappear at the last ecdysis of the nymph but remains, though functionless. Hagen says that in a form he studied the whole structure disappears at that time. Whatever be the case, as far as the final moult is concerned, it is true that in the adult *Plathemis lydia* the rectal breathing apparatus has disappeared. There is indeed no further use for it since the stigmata are then functional.

The tracheal system in the nymph of *Plathemis lydia* differs in most of its details from that of *Æschna cyanea* described by Oustelet, '69. He describes four main trunks extending the length of the body and a pair of smaller abdominal tubes. These correspond to the dorsals, ventrals and laterals in *Plathemis lydia.* The four main trunks supply the rectum and respiratory papillæ in a manner roughly similar to that in *Plathemis lydia.* In *Æschna cyanea,* however, the rectal tracheæ arise from the hinder part of the dorsal at regular intervals, while in *Plathemis lydia* the hind part of the dorsal abruptly breaks up into many fine branches. The rectum of the former is furnished with respiratory papillæ, while the latter has flattened leaf-like gills. In both the tracheoles are present in loops.

In agrionid nymphs there are three long, flat caudal processes thickly supplied with tracheæ, and serve as an apparatus for taking the air from the water into the tracheal system. They are morphologically identical with the caudal spines in *P. lydia.* In the nymph of *Calopteryx*, an agrionid, there are a few internal rectal gills in addition to the three long caudal gill processes. In these forms the gill apparatus is mostly exterior. In the Æschnidæ the branchial apparatus is within the rectum. The inside surface of the rectum is provided with six longitudinal bands, each bearing a double row of folds or papillæ. In the Libellulidæ, of which *Plathemis lydia* is a member, we find not papillæ but flat processes or gills.

In the Libellulidæ there is found the highest form of the rectal gill breathing apparatus, the most specialized branchial arrangement of these three groups. On the other hand the Agrionidæ have the simplest form while the Æschnidæ have the form of branchiæ intermediate between the other two. If we were to arrange these families according to branchial complexity we would have the following order: (1) Agrionidæ, (2) Æschnidæ, (3) Libellulidæ. And this is the order in which they are arranged by systematists. It merely indicates that the tracheal system is correlated in its complexity with the complexity of other parts, and corroborates Lubbock's statement that the tracheal system conforms to the contour of parts.

As to the phylogenetic origin of the branchial apparatus very little is known. Chun, '75, stated that Leydig had described in *Phryganea grandis* a structure which indicates that the rectal branchiæ of Libellulidæ might be considered as being developed from the rectal glands of other insects. Sharp, '95, after careful examination of Leydig's work failed to find such reference. Chun and others, however, have regarded this as the probable origin of branchiæ. Sadones, '95, after a careful examination of the evidence concludes by saying that Chun and the others who hold the above views persist in "perpetuant une notion erronée des anciens anatomistes."

In the nymph of *Plathemis lydia* the pair of thoracic stigmata between the pro and mesothorax are practically closed. When the nymph is placed in a thick solution of glycerine it invariably comes to the surface and protrudes the posterior end of the abdomen out of the fluid, the anal valves opening and closing vigorously. The caudal end always protrudes, indicating that the stigmata are not functional as yet. Hagen, '80, on the other hand believes that the thoracic stigmata are functional in the nymph. The best evidence is furnished by Dewitz, '90, who conducted a series of experiments. When the older nymphs of *Æschna* were placed in alcohol bubbles escaped from the thoracic stigmata. But in immature nymphs no gases escaped though these were subjected to a much severer test. The transition then from rectal gill breathing of the nymph to the open stigmatal type of the adult is not as sudden as ordinarily supposed, but that as the nymph grows older it gradually changes from gill breathing to the open stigmata of the adult.

When one recalls that the fine tracheoles to the rectal gills originated ultimately from the dorsal and ventral tracheæ and that these are connected posteriorly and anteriorly then it appears clear that it is hardly correct to assign the carrying of pure air to one set of tracheoles in the gill and the carrying of impure gas to the other set of tracheoles. A detailed examination of the tracheal system demonstrates not only its complexity but its many interconnections and therefore its unity. It may be regarded as the lungs of the animal whose branches ramify throughout the body, present in every tissue, carrying oxygen to every cell directly and returning the products of respiration from the tissues to the exterior. It is an arrangement admirably adapted to an organism leading a very active life — much more active than that of many of the higher forms.

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OBSERVATIONS ON THE PROGENY OF VIRGIN ANTS.

ADELE M. FIELDE.

The value of the experimental work here presented lies mainly in the complete protection of the virginity of the ant-mothers during their whole lifetime, or from their pupa-stage to the close of the experiments undertaken with them, and in the perfect safeguarding of their eggs from contact with spermatozoa outside the body of the ant.

At the present time, so far as is known to the writer, published observations on the offspring of worker ants may be placed in three categories. Those in the first category present a possibility that a queen's eggs were inadvertently included in the nest with the workers sequestered. When ants are transferred from a natural to an artificial nest, it often happens that eggs, unobserved at the time of sequestration, are discovered in the new nest within a few days thereafter. Ants are tenacious of their charges, and they sometimes conceal eggs or small larvæ in their mouths, or carry them adhering to their persons. In this way eggs may be unwittingly transferred to the new abode and may afterward be brought together in a pile or packet, the observer believing them to be the product of the worker-ants when they are really the issue of a queen in the old habitation. Unless the ants were singly and carefully examined and freed from adherent eggs, or unless a longer time than the twenty days ordinarily required for incubation has elapsed since the segregation of the workers, there is reason for suspecting that the eggs may have been deposited by other ants than the sequestered ones.

In a second category may be included all those cases in which larvæ were intentionally introduced among the segregated ants. Such larvæ may not have reached the pupa-stage sooner than the issue from eggs deposited in the new nest, and it is impossible to maintain that the older and the younger larvæ are always distinguishable. I have had, in my artificial nests, larvæ of *Cremastogaster lineolata*, scarcely larger than the eggs from which they emerged, remaining for many months without visible growth, and then developing simultaneously with the issue of eggs deposited a half year later. I am informed by Dr. J. H. McGregor that larvæ of *Camponotus americanus* have remained such during ten months in his artificial nests at Columbia University; and by Dr. I. A. Field that larvæ of *Camponotus pennsylvanicus* remained under his observation in apparent good health and without visible growth for nearly fifteen months. Admitting that certain eggs were deposited by workers, we still lack assurance that introduced larvæ were not mingled with their issue, and thus there is created a reasonable doubt as to the origin of the callows appearing in the nest at a later date.

To a third category we may relegate the numerous accounts, including my own, concerning the offspring of workers that had previously lived with males, and also those accounts in which male ants were hatched and permitted to remain within the segregated group of workers. We know that ants sometimes mate within the nest, and we have the results of many dissections indicating the capacity of certain workers for impregnation. Miss Holliday[1] found not only ovaries but a seminal receptacle in certain individuals representing three genera of ponerine, two genera of myrmicine, and one species of camponotine workers, none of whom was externally distinguishable from its fellows. We can no longer consider the workers of all species of ants as sterile females. In view of the evidence that among workers, showing no difference in external structure there have been found, in numerous species, many members with both ovaries and seminal receptacles; and of the testimony of competent witnesses that the male ants sometimes pursue the workers with an ardor equal to that shown in their pursuit of the queens, we must abandon the long cherished notion that eggs deposited by worker ants are always unimpregnated.

We do not know even that ant-eggs may not be fecundated outside the body of the female, and this possibility should not be ignored in cases where the egg-piles are traversed by mature males in pursuit of queens and workers.

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[1] "A Study of some Ergatogynic Ants," Margaret Holliday. Contribution from the Zoölogical Laboratory of the University of Texas, June, 1902.

The absence of indubitable proof that the unfecundated ant-egg produces either male or female ant impelled me to undertake the formation of ant-groups in which no member had ever lived with a male, and from whose abode males were excluded. In the summer of 1904 I sequestered pupæ, with two or three workers to take care of them, in artificial nests in which there were neither queens nor males, and as soon as these pupæ hatched I segregated the callows in new nests into which no egg, larva or pupa was ever introduced. My ant-groups were thus made up of workers indubitably virgin, and the eggs deposited in their nests were certainly unimpregnated.

GROUP A. *Camponotus pictus.*

Group A consisted of thirty workers, majors and minors, of *Camponotus herculeanus pictus*, hatched between July 11 and 31, 1904, and kept in segregation from their hatching until October, 1905. Their first eggs, ten in number, were deposited between May 14 and 18, 1905, and these had increased to about fifty on June 4. The first larva appeared on June 6, nineteen days after the first eggs were observed. On July 7 the larvæ had made notable progress in number and in size, the largest then being as long as an adult worker. The first cocoon was spun on July 16, and the first offspring of these segregated virgin workers appeared on August 14. Between August 14 and September 30 their cocoons gave forth thirty-two notably large and sturdy males. That no female might escape observation if hatched from these cocoons, the cocoons were transferred, soon after their formation, to an annex of the nest where only five workers were admitted. Cocoons and nurses were daily counted, and it is certain no queen nor worker ever hatched from these cocoons.

GROUP B. *Formica argentata.*

Group B consisted of about fifty *Formica argentata* workers, all hatched from sequestered cocoons during September, 1904, and kept in segregation by me from the time of hatching until after the close of this series of observations. From eggs deposited on July 7, 1905, the first larva appeared on July 19. The first cocoon was spun on August 4, and the first ant hatched on August 31. Only five cocoons were formed in this nest, and each of these rendered a large, fine male.

DR. FIELD'S GROUP C. *Formica argentata.*

Dr. Irving A. Field, who simultaneously with myself, sequestered pupae from the same wild west that provided my B group, likewise segregated virgin workers, to the number of one hundred and twenty-five, all hatched between August 20 and September 23, 1904. From eggs laid between June 6 and 13, 1905, the first larva appeared on June 21; the first cocoon on July 23; and the first callow on August 7. All the young produced in this group were males, of which fourteen had appeared before September 3, 1905.

DR. FIELD'S GROUP D. *Formica pallide-fulva fuscata.*

This group, while failing to meet prescribed conditions in so far as the workers were not sequestered during the whole of their lives, is herein inserted because its history is clearly recorded. It consisted of many workers, secured by Dr. Field, at Middlesex Fells, Mass., on March 15, 1904. The ants were frozen in a mass when taken from the ground, and every ant was so carefully examined before her insertion into the artificial nest that there is no probability that eggs were introduced into the segregated group of workers. This group remained under Dr. Field's observation at Harvard University. It was placed in a chamber having a temperature of from 75° to 85° F. or from 23° to 30° C. On March 20, five days after the sequestration, the first egg was laid; on April 4, the first larva appeared; and on April 17, the first cocoon was spun. Before June 10, forty-six males had appeared in this nest; and no other than male young had been produced.

GROUP E. *Cremastogaster lineolata.*

The progeny of a queen ant whose life-experiences have all been under observation, is believed to be here for the first time enumerated.

On August 18, 1903, a queen *Cremastogaster lineolata* hatched in a sequestered group of pupæ in one of my artificial nests[1] and was immediately removed to a small nest, where there were several newly hatched workers and males of *Stenamma fulvum*, this group being originally created for observation of the behavior of a queen having a family made up of ants of other species than her own. When the queen was but a few days old, I clipped off her wings to secure her greater safety from accident among the viscid food-stuffs in the nest. During the ensuing year, all the males died, and three more were hatched from eggs deposited by the *Stenamma* workers. I did not expect this queen to lay eggs, because I had previously kept unmated queens (of *Stenamma fulvum*) a whole year without their losing their wings or depositing an egg during that period. Dr. McGregor also kept winged queens (of *Camponotus americanus*) ten months without their losing their wings or depositing eggs.

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[1] My ants were under my care at the Marine Biological Laboratory at Woods Holl, Mass., during the summers, and at my home in New York City during the remainder of the year.

On July 31, 1904, I removed all the *Stenammas* from this nest, cleaned it thoroughly, and gave to the queen forty newly hatched workers from her own colony, probably her own sisters. Three days later there were ten eggs in the nest, and on August 21 there were more than three hundred. That these eggs had been laid by the queen was indicated by their size and by the immaturity of all the workers in the nest. The queen was eight millimeters in length, the workers only three to four millimeters. Moreover, I compared the eggs with those of an isolated queen, *Cremastogaster lineolata*, and found them to match precisely.

Young larvæ were first observed among the eggs on August 28; the first pupa appeared on December 22, 1904, and two males hatched on January 10, 1905. These, when a day old, were transferred to Dr. W. M. Wheeler for expert examination concerning signs of hybridization, and were by him reported to be typical *Cremastogaster lineolata* males. Their successors in the nest were like them. No male was permitted to mature in the nest, all except the first two being removed before hatching. Before the end of September, 1905, sixty-three males had been produced in this nest, the offspring of this virgin queen. No young queen or worker had been seen, though the pupæ in this species are always naked, and the young had been carefully examined at least twice a week.

While this *Cremastogaster lineolata* virgin queen was producing male offspring only, a queen, *Camponotus pennsylvanicus*, living in a similar nest of mine, supplied with the same food, subject to the same daily temperature, and having about the same number of worker-servants, produced numerous offspring, exclusively female. The *Camponotus* queen had been captured when deälated, presumably after her mating. Although these queens were of different subfamilies among the ants, the similarity in all the conditions of their environment except the incident of mating, points to a probability that the sex of their respective progeny was determined thereby, unimpregnated eggs producing males, and impregnated eggs producing females.

It is an interesting fact that during the twenty-six months that this *Camponotus pennsylvanicus* queen remained under my observation no male appeared among the many tens of her offspring; while eleven of her segregated daughters in the care of Dr. Field produced at least three male, and no female, offspring.

The observations here recorded establish the view that some virgin workers lay eggs, and that many ant-eggs that have had no contact with spermatozoa produce males. Not until female progeny shall have been observed to issue from eggs protected as were those of my *Camponotus pictus* should we consider the Dzierzon theory inapplicable to ants. No ant indisputably virgin, with her eggs perfectly safeguarded from spermatozoa, has yet presented evidence against the extension of this theory to the Formicidæ.

Marine Biological Laboratory,
Wood's Holl, Mass., September 1905.

TEMPERATURE AS A FACTOR IN THE DEVELOPMENT OF ANTS.

WITH FURTHER OBSERVATIONS ON ANTS DEPRIVED OF FOOD.

ADELE M. FIELDE.

Some recent experiments made by me lead to the conclusion that temperature is a dominant factor in the development of ants; that, other things being equal, it determines the time of the deposit of the eggs, the length of the larval period, and the hour of exit from the cocoon; and that the developing young of unlike species of ants are differently affected by the same degree of heat. It also appears probable that unlike species of ants develop and deposit their eggs at different temperatures, fixed for each species.

The greater activity of the adult ants in higher temperatures, with the increased movement of the anterior end of the larva which may be observed when the temperature rises, and the quickening of the pupæ which occurs in the hottest days, are doubtless an effect of the stimulation of metabolism by heat.

EXPERIMENT A.

My N. queen *Camponotus pennsylvanicus* laid eggs in the first week in August, 1903, which were kept in my living room where the temperature seldom rose above 70° F. or 21° C., and the earliest ant to issue from these eggs hatched on April 25, 1904, when the temperature had risen to 78° F. or 26° C. On July 14, 1904, I removed this queen to a new nest, where she was kept at a temperature that seldom rose above 70° F., and she laid no eggs until after I had, on November 20, 1904, removed her to a room where the usual temperature was from 82° to 85° F., rarely falling to 70° F. and occasionally rising to 90° F. On December 2 I observed twenty eggs, which had increased to sixty on December 11. The first larva appeared on December 18. On December 30 all the eggs had hatched and there were several tens of larvæ. The first cocoon was spun on January 8, and the first callow appeared on January 29, 1905. This indicates about twenty days for incubation, a month for the larval and twenty days for the pupal period, all at a time when the congeners of this queen were merged in winter repose.

This N. queen shared the labors of the workers in the care of the young, which continued to develop until the 26th of March, when I removed the nest to a room having a fairly steady temperature of 70° F. At this temperature the remaining cocoons failed to hatch, and the fifty larvæ ceased to grow. The larvæ did not increase in size till the last days of the following June, after the temperature had risen to 76° F. The first cocoon of this newer brood was spun August 6, 1905.

Although this queen had deposited eggs in March, 1904, she deposited none in March, 1905, nor did she lay any thereafter until July 26, 1905, when she again deposited a few eggs. During the first week in August, 1905, the eggs were increased to about the same number that she had laid at the same season two years previously. Her failure to lay eggs in August, 1904, was doubtless due to the agitation consequent upon her service in several of my experiments at about that time. I have often observed that psychic influences affect the deposit of eggs by ant queens. Her failure to lay eggs in March, 1905, was probably due to exhaustion consequent upon the work to which she had been stimulated by high temperature in the previous December.

Since the food supply, the humidity, and the number of workers were factors whose variation was but slight in the nest of this queen, it appears probable that the time of the development of eggs and the growth of the young was determined mainly by the temperature.

Experiment B.

My *Cremastogaster lineolata* queen laid hundreds of eggs in August, 1904, and the larvæ therefrom grew scarcely at all until, on November 20, 1904, I removed her nest from a room where the usual temperature had been 70° F. to a chamber where the temperature was usually from 82° to 85° F. Early in December the larvæ began to increase in size and on December 22 the first pupa appeared among them. The young continued to thrive and fifty-three pupæ had been developed in the nest before March

26, 1905, when I removed the nest to a room having a usual temperature of 70° F. No more pupæ developed thereafter until late in the succeeding summer. The larvæ remained in a state of arrested development from the end of January until the end of the following June, when, under the influence of the natural rise in summer heat, they entered upon a period of renewed growth and in August presented me with ten more pupæ.

This queen laid eggs in June, when the temperature rose to 75° F., and continued to deposit them at various times throughout the summer.

Experiment C.

A group of thirty workers, majors and minors, of *Camponotus herculeanus pictus* hatched between July 11 and 31, 1904, were kept at the temperature of 70° F. or 22° C. until November 20, when they were removed to a chamber whose usual temperature was from 82° to 85° F. or 28° to 30° C. On March 26, 1905, they were returned to their place in my living-room, where the temperature was usually at 70° F. They laid no eggs until May 14, and then deposited about fifty before June 4, 1905, apparently under the influence of a rise in the temperature to 78° F. or 25° C.

The age of these ants may, however, have had influence on the time of egg-laying. It would be interesting to ascertain, by dissections of worker-ants, whether differences in the degree of development of the ovaries in workers of the same species is correlated with difference of age in the respective workers. It may be that the ovaries of many worker-ants do not develop until some months, or until the second season, after hatching.

Experiment D.

A group of fifty workers, *Formica argentata* hatched during September, 1904, were kept by me at a fairly steady temperature of 70° F., and laid no eggs until after they were removed to a chamber having a usual temperature of from 82° to 85° F. Their first egg was deposited on January 1, 1905, and during the ensuing week the eggs increased rapidly in number, so that on January 8 there were more than one hundred. The first larva was observed on January 15. None of the larvæ reached the pupa-stage, and all these eggs and their issue had disappeared before March 19. On March 26 this nest was removed to a room having a temperature of 70° F., and these ants ceased from egg-laying until the temperature again rose to more than 80° F. in the following July. The young larvæ from the July eggs rapidly disappeared in the intense heat of July, 1905, and only five of them spun cocoons. It seems that the development of the eggs of this species demands a high temperature, while the development of the larvæ requires a lower degree of heat.

Dr. Irving A. Field, keeping a nest of one hundred and fifty worker-ants of this species, at Harvard University, gives me the following account of said nest: "The ants all hatched between August 20 and September 23, 1904. They laid no eggs until after November 30, when the nest was placed in a chamber having a temperature of from 75° to 85° F. Between December 30, 1904, and January 9, 1905, about a hundred and fifty eggs were laid, all of which were subsequently addled or else were eaten by the ants. But between June 6 and 13, 1905, about sixty eggs were laid, and from these, in natural summer temperature, many larvæ safely passed to the pupa-stage."

Experiment E.

I had one group of virgin workers of *Stenamma fulvum* two years old; one group of virgin workers of *Stenamma fulvum* one year old; and one group of virgin workers of *Camponotus americanus* newly hatched, that deposited no eggs during September, October and November, 1904, when they were kept at a temperature of about 70° F. But in all three of these groups, eggs that produced larvæ were deposited between November 20, 1904, and March 26, 1905, when they were kept at a temperature of from 82° to 85° F.; and all were again subjected to a completely arrested development of the young when the temperature fell to 70° F. between the end of March and the middle of June, 1905.

Groups of virgin workers of *Formica Schaufussi* and of *Formica neogagates*, housed, fed, and kept at the same daily temperature as were the above named ants, laid no eggs during either the periods of low or of high temperature, but deposited eggs after the middle of June, 1905, when the summer heat rose to 78° F.

OTHER EXPERIMENTS.

Dr. Field had a nest of workers of *Formica pallide-fulva fuscata* in which many eggs were deposited in March, 1905, when kept at a temperature of from 75° to 85° F. and the issue of these eggs passed safely through the larval and pupal stages at the same temperature. Dr. Field had also a nest of *Camponotus pennsylvanicus* workers who failed to rear their larvæ to pupation in the summer of 1904, but who, under the influence of the above named high temperature brought three to pupation in January, 1905.

Dr. W. M. Wheeler writes to me that *Formica consocians* workers in his artifical nests deposited many hundreds of eggs, in March, 1905, in a room whose temperature never rose above 60° F. or 15° C.

It appears that the time of development may be altered by change of the prevailing temperature and that an intervening period of recuperation will be maintained in spite of a continued temperature-stimulus. Other factors being equal, the development of the eggs within the ovaries, the deposit of the eggs, the feeding and growth of the larvæ, the pupation and the hatching, all appear to be determined by temperature. The degree of heat suiting the species probably varies for the different stages of development.

All the ants involved in my experiments had the same food-supply, the same daily temperature, and similar housing. They were abundantly provided with insect-food and with a variety of sweets. They always appeared to be in excellent health, and hardly any deaths occurred in any of the nests mentioned. It is therefore difficult to refer behavior so varied to other cause than the varying effects of the same temperature on unlike species of ants, or to avoid the inference that in different species the young develop best at different degrees of heat.

Among the ant-young observed by me, none has developed at a temperature below 70° F.; while long exposure to a degree of heat above 90° F. manifestly causes injury. Two minutes exposure to a temperature of 122° F. or 50° C. will kill the most vigorous adult.

FURTHER OBSERVATIONS ON ANTS DEPRIVED OF FOOD.

Among the groups mentioned in my paper on "Tenacity of Life in Ants" (in the BIOLOGICAL BULLETIN, Vol. VII., No. 6, Nov., 1904, p. 300) were seven *Formica subsericea* workers that had been deprived of food three months and remained alive at the time of writing. These seven ants had been picked up from a roadside where they were foraging under a linden tree, on July 3, 1904. They were kept in a Petri cell containing only themselves and a bit of sponge saturated with water. Cell and sponge were cleansed with alcohol at intervals never exceeding four days, until the end of September, after which time the cell and sponge were cleansed only once a week. No growths were at any time visible under a lens magnifying ten diameters.

On February 3, 1905, one of these ants was removed for dissection, after an enforced fast of just seven months. One died on November 27, one on November 29, 1904; one on February 17, one on February 19, one on March 14, and one on March 28, 1905, the latest survivor having lived nearly nine months without food.

A *Camponotus Americanus* worker, under like conditions, lived without food from July 13, 1904, to February 12, 1905, just seven months.

Two sister queens, *Camponotus Americanus*, one deälated, one winged, under the same conditions, lived without food from July 13 to December 6, 1904, both dying on the same day. These queens had been bred in an artificial nest, and may have lacked the stamina of untamed ants.

After the first of October, all these fasting ants were kept at a fairly steady temperature of 70° F. or 21° C.

Until the day of death the ants walked about, reacted normally to light and heat, and gave no evidence of failure in any of the senses.

In spite of the prolonged fast, there was no fighting nor cannibalism in any group of these ants, and the bodies of those that had died were always found to be intact.

The ability of ants to survive a submergence of several days in water explains their persistence in areas where freshets periodically exterminate all other land insects, and their power to live for many months without food explains their occupancy of places subject to long droughts that destroy their whole food-supply.

Marine Biological Laboratory,
Wood's Holl, Mass., September, 1905.

NOTES ON THE VARIATIONS OF RHEGMATODES.

Chas. W. Hargitt.[1]

During the summer of 1900 I collected about two hundred specimens of *Rhegmatodes tenuis* for the purpose of studying its development. But this I was unable to carry out, owing to the inability of keeping the medusæ long enough in the aquarium to secure the eggs, few seeming to bear ripe gonads at the time of capture.

It was observed that many specimens showed more or less variability, and attention was then directed toward a study of the variations exhibited among the various organs. The comparatively few specimens obtained led to the postponement of a final study of the problem, hoping to secure additional material. This has been carefully sought during three subsequent summers, but in vain; only during 1902 have any specimens at all been found, and these very few and immature. The following "Notes" are therefore submitted as a slight contribution to the general problem of variation among Hydromedusæ, a more extended account of which was made by the writer in 1901.[2]

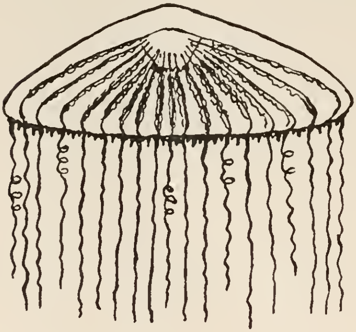

Fig. 1. *Rhegmatodes tenuis.* Semi-diagrammatic.

The general features of *Rhegmatodes* are shown in Fig. 1. The medusa varies in size at maturity from about 50 to 95 mm. in diameter. In younger stages the shape is more or less hemispherical, the tentacles and radial canals very much fewer in number, the earliest stage which I have found having but four canals and four tentacles. With growth the number increases very largely, but they are not correlated as at first, the latter becoming more numerous, besides also varying numbers of tentacular spurs or clubs between the bases of the fully developed ones.

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[1] Contributions from the Zoölogical Laboratory, Syracuse University.

[2] Biol. Bull., Vol. II.

In view of the constantly increasing number of these organs, which are the more prominent organs usually involved in variation, it might seem a very uncertain problem to undertake anything like a critical determination of the quantitive variations. So far as the merely numerical aspects of variation are concerned this is undoubtedly true, as will be seen. I may, therefore, say in the outset that, both in view of the doubt just expressed, as well as in the comparatively small number of specimens available, no attempt will be made to present anything like a statistical account. It will be the purpose, rather, to submit a brief account of variations of a qualititative, or substantive character.

So far as I am aware, the only account of variations in the Family Æquoridæ is that submitted by Claus,[1] concerning *Æquorea forskalii* in a sharp critique of Haeckel's multiplication of species, and enlargement of the scope of the family. Claus calls attention to the striking differences exhibited by young specimens as compared with adults, and also to the almost equally marked differences as to coloration found in specimens of various ages and sexes, as well as to the highly variable character of the number of canals, shape of mouth and oral arms under different conditions of contraction, etc.

In connection with his description of *Rhegmatodes floridana*, Dr. A. Agassiz has merely referred to the fact that in the Æquoridæ, "the chymiferous tubes especially are very irregularly formed. They are frequently added all on one side of the spherosome, and almost fully formed before they begin to develop in the other half."[2]

The matters involved in these observations, while both of importance, hardly touch the more fundamental problems of variation as now understood. The critique of Claus is eminently fitting as a protest against the unwarranted multiplicity of species based upon such flimsy characters as those cited. The matters referred to by Agassiz deal more directly with phases of development than with those of variation, though closely related thereto, as will be seen in some of the illustrations of features to be described presently.

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[1] Arbeit. Zoolog. Inst., Wien, 1881, p. 283.

[2] "North. Am. Acalephæ," 1865, p. 97.

In view of the difficulty in accurately studying the otoliths of preserved specimens no attention has been paid to that feature, except incidentally. Something of a similar sort is true concerning the matter of variation in tentacles. They are so subject to mutilation and loss, to inequalities of regeneration, etc., that these also have not been given special attention in connection with this species. Chief attention has been directed to the radial canals, and to a less extent the gonads.

ASYMMETRY.

In a few cases marked asymmetry was apparent. In Fig. 2 is shown a condition found in several specimens. In the particular one from which this sketch was made the medusa had 24 radial canals, 12 of which were within a single quadrant of the umbrella, the others being about equally distributed over the other three fourths of the body. As will be observed, several of these canals were incomplete, as were also the associated gonads, the latter appearing to be either rudimentary or tending to degenerate. A somewhat similar condition is shown in Fig. 8; where in addition to the asymmetry, and incompleteness of several of the canals, there is also a fusion of the terminal portions of some of the canals.

The earlier citation of the account of Agassiz concerning the very "irregularly formed" condition of certain of these canals during development is significant in this connection. It would seem as if the condition involved in the case from which this illustration was drawn might be of a similar nature, though in the present instance, it must be remembered, that we are dealing with an adult and not a young specimen. It may not be improbable that in certain instances these apparent irregularities found during development may persist as permanent conditions in the adult.

RADIAL CANALS.

Attention has been already directed to the fact that the canals increase in number with the growth and age of the medusa. No attention need be taken therefore of this feature in relation to variation, though when it is remembered that in the vast majority of all Hydromedusæ the radial canals are normally but four in number, the question naturally arises as to the conditions under which this extraordinary increase has arisen, and what it may signify. Such an inquiry would, however, lead too far afield for our present purpose. Mayer has raised and briefly discussed the problem in connection with a study of "The Variations of a Newly Arisen Species of Medusa."[1] In this paper Mayer suggests that one of the ways in which a pentamerous medusa may may have arisen from an ordinary tetramerous form is by bifurcation of one of the canals, which, if it became hereditary, would easily account for the condition of pentamerism.

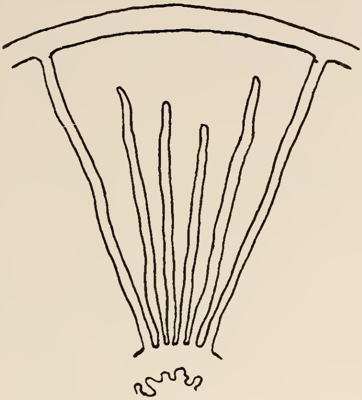

FIG. 2. Showing series of imperfect canals.

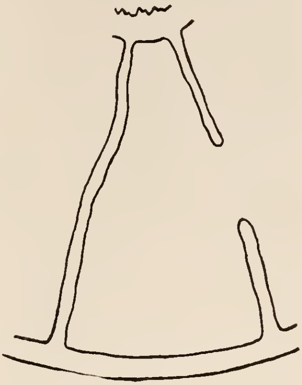

FIG. 3. Showing central and peripheral development of radial canal.

The present writer has discussed somewhat the same general problem in an earlier paper (*op. cit.*, p. 239). If one had only a pentamerous or hexamerous condition for which to find some simple explanation, that just suggested might appear adequate. But when we have conditions like that of *Rhegmatodes*, not to mention many others of a similar sort, it will be more or less clear that such an account would be inadequate. It would seem far more probable that these supernumerary canals have arisen under the appropriate stimulus entirely independent of any special phyletic relations.

[1] "Variations in a Newly Arisen Medusa." *Bull. Brooklyn Inst. Arts and Sciences*, Vol. I.

In Figs. 3–5, as well as in those already cited, are shown interesting and more or less common forms of variant canals. Similar conditions are also to be distinguished in several of the other figures given. For convenience we may discuss these features under the following heads: (1) Bifurcations, (2) loops, (3) spurs, (4) anastomoses.

It may be altogether probable that these conditions graduate somewhat insensibly into each other, so that they may really be but varying expressions of one and the same fundamental process But as suggested, such a classification will at least serve as a convenience in description.

1. *Bifurcations.* — Several illustrations of this feature have already been cited. Others are shown in Figs. 4–6. For the most part these apparently arise as a result of division of the peripherally developing canal, though the centripetally directed type is not lacking, as is seen in Fig. 4. It may be said in passing. that the figures given are for the most part typical ones, each representing usually several similar cases. In development the canals usually arise at the gastric pouch and grow toward the periphery. Agassiz (*op. cit.*) has said upon this point, "Additional chymiferous tubes in the Æquoridæ are developed from the digestive cavity, as shown by Kolliker, and not from the vertical tube, as in the case of the branching tubes of *Willia.*" This I have found to be the case in a study of the very young medusa. On

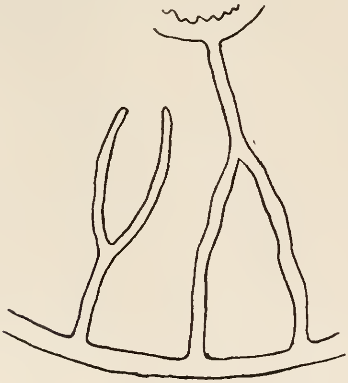

FIG. 4. Showing centripetal and centrifugal canals with bifurcations.

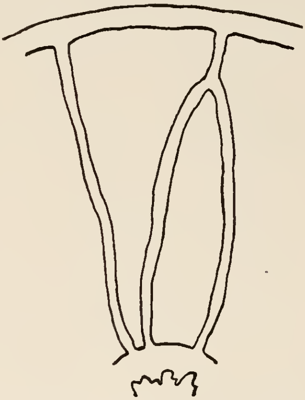

FIG. 5. Showing union of peripheral portions of two canals.

the other hand, it must not be overlooked that in the Geryonidæ we have for the most part the opposite process, namely, the tubes arising from the marginal canal and developing centrally. Then in Figs. 4, 5 and 6, we have conditions which seem to show undoubtedly that such has been the mode of origin and development in *Rhegmatodes*. It brings before us, therefore, a form of variation at once morphological and physiological, or perhaps better, embryological.

2. *Loops.* — In Figs. 7 and 8 are shown what has seemed best described as loops. These seem to have resulted from the fusion of two or more canals whose terminal portions have approximated each other and finally become confluent. These were more or less common conditions among these medusæ. In my earlier paper (*op. cit.*) I have figured and described very similar features in *Gonionemus*, and Agassiz and Woodworth have likewise described similar features in the genus *Eucope*.[1] I incline to doubt whether any particular significance may be said to attach to such variations as these. It is certainly hard to discern any particular selective value they might have in the betterment of the species. It seems rather to be illustrative of those types of indefinite or fluctuating variations which abound more or less throughout the organic world, but having little or no bearing or significance in natural selection.

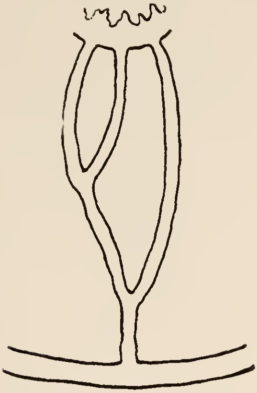

Fig. 6. Showing varying anastomoses.

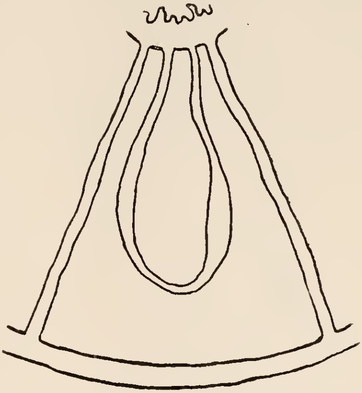

Fig. 7. Showing loop resulting from confluence of distal portions of adjacent canals.

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[1] "Variations in the Genus *Eucope*," *Bul. Mus. Comp. Zool.*, Vol. XXX.

3. *Spurs.* — These features are among the most common of the variant phenomena associated with the radial canals. Very few medusæ fail to exhibit something of the sort in greater or less degree. They are, however, more than usually abundant in *Rhegmatodes.* In a forthcoming paper on "Variations Among the Scyphomedusæ"[1] I call attention to similar conditions associated with the abundant anastomoses which occur in species of *Aurelia.* As shown in several of the accompanying figures, especially 9 to 12, they probably have some such relation in *Rhegmatodes.*

4. *Anastomoses.* — These are features, as just suggested, involving to some extent the preceding, and almost as common, especially in *Rhegmatodes.* Figs. 10 to 14 are a few illustrations of a condition found in many specimens, both small and large. Whether a similar condition may be found to occur in other Æquoridæ I do not know, or whether similar features exist in other of the Hydromedusæ having supernumerary canals is apparently unknown. When compared with the complex canal system found in the genus *Aurelia* it would at once suggest the query as to whether it might imply any phyletic relation with these medusæ. But this does not seem to me to be the case. It has probably only a more or less indefinite correspondence in the most general way, barely enough likeness to suggest a remote parallelism, but nothing of greater significance. Furthermore, as suggested in the previous section, it is difficult to perceive anything like a definite variation in a given direction, tending toward the establishment of a type of hydromedusa with a complicated and correlated canal system such as that of *Aurelia.* And while the total of these variational features reached as high as 27 per cent., which is but slightly higher

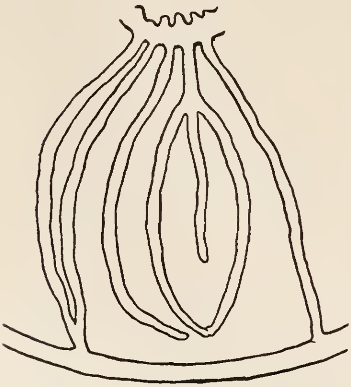

FIG. 8. Showing variously branched and united canals.

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[1] "Variations Among the Scyphomedusæ," *Jour. Exp. Zool.*, Vol. II.

than the ratio I have found in *Aurelia* (*op. cit.*), it may still be doubted whether it sustains any direct selectional value to the species, any more than in the case of *Aurelia*.

GONADS.

The gonads are closely correlated with the radial canals, forming a double fold along the line of each canal. In not a few cases it was found that the gonads of certain canals were more or less rudimentary. Whether this may have been due to arrested development, or to some wholly unrecognized cause it is impossible to say. As a rule, it may be said that in those canals marked by incompleteness, as in Figs. 2, 3 and 4, the gonads were of this character, *i. e.*, showed signs of arrested development, or atrophy. An apparent exception was observed in the case shown in Fig. 7, where in the loops formed the gonads were larger and better developed than those of the normal canals of the same specimen. I think an explanation of this condition may be found in the fact that here the short loops of the canals were in closer connection with the gastric pouch, and thus provided with a larger food supply than in either of those of such incompleteness as shown in Figs. 2–4, or indeed the normal canals. Aside from these rather incidental aspects of variation there was little to call for special emphasis, or fuller details.

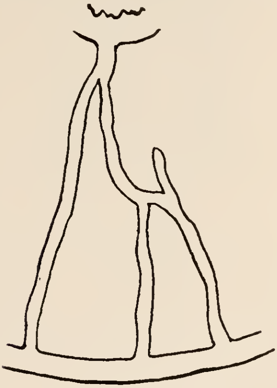

FIG. 9. Showing variations in branching, etc.

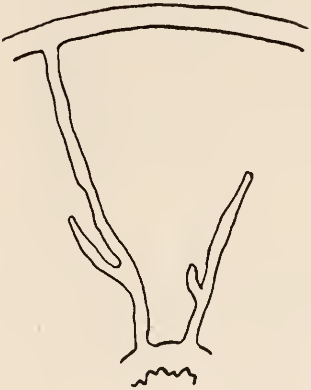

FIG. 10. Showing spurs.

Tentacles.

As intimated in the outstart, there has been given but slight attention to variations in these organs. The tentacles of *Rhegmatodes* are both numerous and extremely delicate. It is therefore, difficult to distinguish numerical variations, or irregularity in position. I shall call attention only to one feature, concerning which, however, there is little to be said, the occurrence of branching, or bifurcation. This is a fairly common feature in many medusæ, and in my earlier paper I have figured and described numerous cases. Agassiz and Woodworth (*op. cit.*), have also described several cases among the Eucopidæ. Though somewhat careful attention was directed to this in *Rhegmatodes* anything of the sort is extremely rare. Only a single instance of what seemed to be a basal bifurcation of a tentacle was detected, and that was not so evident as to be absolutely certain.

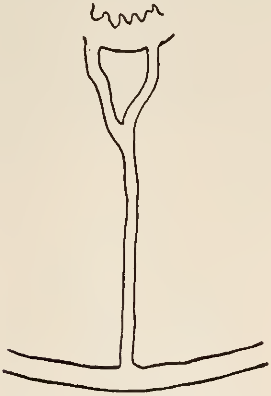

Fig. 11. Showing union of basal canals in a single one.

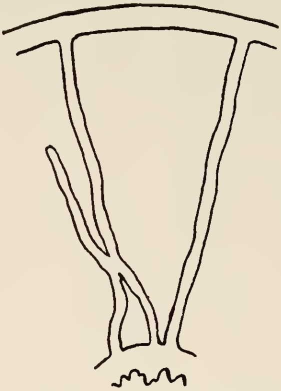

Fig. 12. Showing anastomosis and imperfect canal.

As a final word upon this feature it may be stated that as a rule the number of tentacles was usually greater as the specimens increase in size. To this, however, there were many exceptions. For example, one specimen measuring 47 mm. in diameter had 66 tentacles, while another which measured but 36 mm. in diameter had 69 tentacles. This may be taken as somewhat typical of many similar cases. In the paper of 1901, already cited, I have called attention to numerous instances of a similar sort in *Gonionemus*. But as said in another connection, I do not attribute any particular significance to this feature in species like these, in which the numbers are large.

OTOCYSTS.

The limited attention directed to these organs gave very much the same results as in the case of tentacles. Ordinarily they are said to occur in a more or less regular order between the bases of the tentacles; and in some species this order is very exact, for the most part. But in *Rhegmatodes*, as in *Gonionemus* (*op. cit.*), this does not seem to be the case. While difficult in preserved material to determine in many cases the presence or absence of these minute bodies with exactness, still a careful search in many of the most favorable specimens failed to detect anything like a definite and symmetrical distribution of them. Agassiz (*op. cit.*) has described a single one between the bases of contiguous tentacles. In many cases two were found, and in a few cases even more. But as intimated above, both the difficulty of their determination, and the great liability of loss of tentacles make the matter of a dogmatic statement on this point unwarranted.

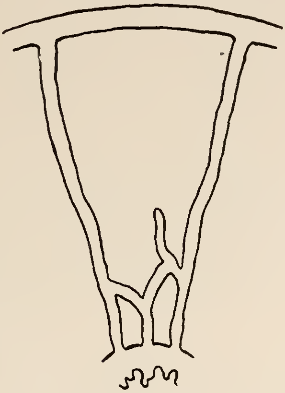

FIG. 13. Showing anastomoses.

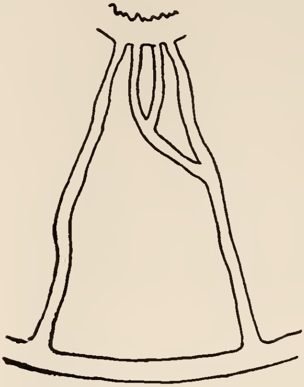

FIG. 14. Showing loops and anastomoses.

SYRACUSE UNIVERSITY,
September 9, 1905.

ON SOME FACTORS GOVERNING THE PERMEABILITY OF THE EGG MEMBRANE BY THE SPERM.

H. H. NEWMAN.

In the summer of 1903 Loeb[1] succeeded, by slightly increasing the alkalinity of sea water, in fertilizing the eggs of the sea-urchin (*Strongylocentrotus purpuratus*) with the sperm of the 20-rayed starfish, with that of Asterias and with that of the Holothurian, *Cucumaria*, while at the same time the eggs were rendered immune to their own sperm.

The question arose as to the character of the change in the egg or in the sperm that makes the former sometimes permeable to its own sperm and impermeable to foreign sperm and sometimes impermeable to its own sperm but quite freely permeable to the sperm of other species.

In one place Loeb tentatively suggests, "that the whole effect of the alkali is simply to bring about a slight physical change in the condition of the protoplasm of the egg, or the surface of the spermatozoön, which facilitates the entrance of the latter into the egg"; and in a continuation of the same series of experiments,[2] published some months later, he suggests "that the main variable determining the entrance of the spermatozoön into the egg may be of the nature of surface tension or a function of the latter."

In the following year Morgan[3] published a paper on "Self-Fertilization Induced by Artificial Means," in which he gives an account of some experiments performed on the eggs and sperm of several tunicates.

Under normal conditions the eggs of one individual cannot be fertilized by its own sperm, but, by treating the eggs and sperm with certain amounts of ether, he was able to get large percentages of self-fertilizations.

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[1] Loeb, University of California Publications, Physiology, Vol. I., No. 6, pp. 39–53.

[2] Loeb, University of California Publications, Physiology, Vol. I., No. 11, pp. 83–85.

[3] Morgan, *Journal of Experimental Zoölogy*, Vol. I., No. 11, 1904.

Among the possible explanations of this phenomenon Morgan suggests "that the surface tension of the egg is of such a sort that it excludes the sperm of the same individual, but this idea does not appear to give a satisfactory solution, for, aside from the fact that it is difficult to imagine how such a relation could exist, there would also occur cases in which the surface tension of the eggs of other individuals would exclude certain sperm, and this would not appear to be the case. It is true that the addition of the ether to the water may cause a difference in the surface tension of the egg, and it might be made to appear that this was the way in which the self-fertilization is effected in ether solutions, but I cannot believe that this is the explanation, because other experiments show that a considerable amount of ether is necessary to cause self-fertilization."

In a later paper,[1] dealing with the same tunicates, Morgan corrects his previous observations to some extent and shows by a series of experiments that "it is not true that the sperm of a given individual will fertilize equally well the eggs of all other individuals."

As a matter of fact his tables show that the sperm of some individuals is wholly ineffective on the eggs of other individuals, and that all degrees of effectiveness are found. Thus his later experiments serve to offset his chief objection (quoted above) to the idea that surface tension might be an effective factor in controlling the entrance of the sperm into the egg.

Morgan showed that the addition of certain amounts of ether to sea water made the sperm more active, and he inferred from this fact that the activity of the sperm was the factor governing fertilization. On the other hand, he states that eggs have been fertilized by very inactive sperm. This seems to indicate that the activity of the sperm is a subordinate factor in fertilization. Moreover, no matter how slow-moving the sperm might be, some would undoubtedly be able to reach some eggs. The failure to fertilize would not then be due, I believe, to a failure of the sperm to reach the egg-membrane, but rather to a failure to fuse with and penetrate the latter. The fusion, as both Loeb and Morgan have suggested, may very readily be dependent upon surface tension.

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[1] Morgan, BIOLOGICAL BULLETIN, Vol. VIII., No. 6, 1905.

At the suggestion of Dr. A. P. Mathews I took up this problem to see whether the surface tension, or the condition of the surface of the egg was of importance in determining fertilization. The working hypothesis was that the surface of the egg possessed a certain definite tension that rendered it permeable to sperm of equally definite surface tension. If the tension of either the egg or sperm is altered their fusion might no longer be possible, although the sperm of other species that might chance to possess a more appropriate surface tension might enter.

If the egg be a colloidal system with a membrane of a certain definite surface tension it should be possible so to alter this tension by the use of certain salts, that the sperm of the same species can no longer enter, and after so doing, to reverse the process and restore the original surface tension and the consequent permeability.

According to Mathews[1] the surface tension of colloidal particles or particles in suspension may be increased or decreased by means of salts acting through their tension coefficients. The egg may be regarded as a particle in suspension of which the membrane is electro-negative. It should be possible to increase its surface tension by neutralizing these charges either wholly or in part. A list of the tension coefficients of salts is given in Mathews' paper on the precipitation of colloids and at his suggestion I tried the alkaline earth chlorides, and particularly calcium chloride, as a salt which should increase surface tension, and sodium sulphate as a salt which should have an opposite effect on the surface of the egg.

The eggs of the minnow, *Fundulus heteroclitus*, were chosen for the experiment. These eggs are almost immune to changes in the osmotic pressure of the medium. It was noticed at the outset that eggs of *F. heteroclitus* became incapable of fertilization by their own sperm after exposure to sea water for periods in excess of an hour or in some cases an hour and a quarter. It seemed possible that the cause of this loss of susceptibility to fertilization might be due to certain salts in the sea water. This matter is partially cleared up by what follows.

The first experiments were undertaken to determine which salts were most powerful in shortening the period of susceptibility of the egg to its own sperm.

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[1] Mathews, *Ameri an Journal of Physiology* XIV., 1905, p. 203.

Experiment I. — Large numbers of eggs from various females were stripped into distilled water, mixed thoroughly and transferred to dishes containing respectively 100 c.c. of

A. $\frac{5}{10}$ *n.* $CaCl_2$,
B. $\frac{5}{10}$ *n.* $MgCl_2$,
C. $\frac{6}{10}$ *n.* NaCl,
D. $\frac{6}{10}$ *n.* KCl.

These solutions were made approximately isotonic with sea water so as to eliminate the factor of osmosis, although, as stated, these eggs are practically immune to osmotic changes. After periods of five, ten, twenty and thirty minutes' exposure to these solutions, successive lots of eggs — averaging about fifty in number — were taken out, washed in sea water and an attempt made to fertilize them in sea water.

The results of this experiment follow:

A. ($\frac{5}{10}$ *n.* $CaCl_2$). No eggs fertilized at all and all showed coagulation of the blastodisc or plasmolysis of the entire protoplasmic mass, after an exposure of five minutes or longer.

B. ($\frac{5}{10}$ *n.* $MgCl_2$). No eggs fertilized and all showed a vacuolated condition of the protoplasm and a considerable amount of plasmolyses after exposures of five minutes or longer.

C. ($\frac{6}{10}$ *n.* NaCl). About 30 per cent. of all eggs fertilized and cleaved normally and nearly all of these formed embryos, even after the maximum exposure of thirty minutes.

D. ($\frac{6}{10}$ *n.* KCl). From 60 to 75 per cent. of eggs fertilized and cleaved normally, producing normal embryos, even after the maximum exposure of thirty minutes.

From this experiment it will readily be seen that calcium salts are the most active in preventing fertilization, while sodium, and especially potassium salts, have relatively little inhibiting effect.

Experiment II. — In order to test the relative toxicity of these solutions before and after fertilization the following simple experiment was undertaken: A large number of eggs were fertilized in sea water, washed rapidly in distilled water and transferred to the same solution used in Experiment I. After standing in these solutions for thirty minutes they were returned to sea water and allowed to develop. In every case nearly all eggs developed normally and formed embryos.[1]

It is worthy of note, then, that the egg becomes immensely more resistant to the toxic action of these salts after fertilization.

Several repetitions of Experiment I. showed an invariable order of activity from the most active to the least active — $CaCl_2$, $MgCl_2$, NaCl, KCl. It seemed advisable to include several other chlorides of these groups to see if their activity in inhibiting fertilization would fall in line with the theory of solution tension.

Experiment III. — A large number of eggs were stripped into distilled water, stirred and distributed into seven dishes containing:

A. $\frac{6}{10}$ *n.* LiCl,
B. $\frac{6}{10}$ *n.* NaCl,
C. $\frac{6}{10}$ *n.* KCl,
D. $\frac{5}{10}$ *n.* $MgCl_2$,
E. $\frac{5}{10}$ *n.* $CaCl_2$,
F. $\frac{5}{10}$ *n.* $SrCl_2$,
G. $\frac{5}{10}$ *n.* $BaCl_2$.

All eggs were allowed to remain in these solutions for five minutes, then washed and an attempt made to fertilize them in sea water, abundance of sperm being added in each case. It was found that so few of the eggs cleaved that a percentage basis could not be employed for all of the salts, yet it was quite evident that LiCl had an action between NaCl and $MgCl_2$, while $SrCl_2$ and $BaCl_2$ fell between $MgCl_2$ and $CaCl_2$.

Evidently the concentration of the seven salts used in this experiment was too great so it seemed advisable to determine the minimum concentration and the minimum time of exposure required by the most active of these salts — $CaCl_2$ — for complete inhibition of fertilization. The following experiment enabled me to determine this.

Experiment IV.—Solutions of $\frac{1}{10}$ *n.*, $\frac{2}{10}$ *n.*, $\frac{3}{10}$ *n.* and $\frac{4}{10}$ *n.* solutions were made up and lots of eggs were exposed in each concentration for five, ten, twenty and thirty minutes before fertilization was tried. It was found that a $\frac{2}{10}$ *n.* $CaCl_2$ solution, after acting for five minutes allowed only 0 to 4 per cent. of eggs to become fertilized. This concentration and time of exposure should give a good percentage basis for estimating the activity of the seven salts used with reference to $CaCl_2$.

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[1] See Mathews, *American Journal of Physiology*, X., 1904, p. 241.

Experiment V. — The same seven salts used in Experiment III. were used in $\frac{2}{10}$ *n.* solutions. The same method of procedure was used throughout as in Experiment III. and the results are given in the following table. The experiment was performed twice and the figures are given side by side for the sake of comparison.

		No. 1.			No. 2.		
	Salt.	No. of Eggs Used.	No. of Cleavages.	Per Cent. of Cleavages.	No. of Eggs Used.	No. of Cleavages.	Per Cent. of Cleavages.
A.	LiCl	72	41	57	38	25	68
B.	NaCl	80	64	80	34	26	76
C.	KCl	92	80	89	45	39	87
D.	$MgCl_2$	78	14	18	50	9	18
E.	$CaCl_2$	108	4	3.7	38	2	5.2
F.	$SrCl_2$	88	15	17	42	6	14.3
G.	$BaCl_2$	82	9	10.9	43	4	9.3

Averaging the percentages of cleavages in the two experiments we find that the salts arrange themselves as follows:—$CaCl_2$, 4.4; $BaCl_2$, 10.1; $SrCl_2$, 15.6; $MgCl_2$, 18; LiCl, 62.5; NaCl, 78; KCl, 88. This corresponds exactly with the order of the precipitating power of these salts on colloidal egg albumin and possibly with the order of the solution tensions of these cations.

These results seem to indicate that the cause of the inhibition of fertilization is of the nature of a precipitation of the colloids of the egg membrane. In order to demonstrate the possibility of rendering the egg impermeable to sperm of the same species and then reversing the coagulation so as to restore its permeability, a $\frac{2}{10}$ *n.* $CaCl_2$ solution was used as a precipitating or coagulating agent and a *m*/10 Na_2So_4 solution was used as a salt which theoretically should reverse the coagulation.

Experiment VI.—A lot of eggs were stripped into 100 c.c. of $\frac{2}{10}$ *n.* $CaCl_2$ solution and after a five minute exposure were divided into two dishes, one containing *m*/10 Na_2So_4 and the other containing sea water as a control. The control eggs were immediately mixed with an abundance of sperm, which was allowed to remain in the dish, but not a single egg cleaved. The eggs transferred to the *m*/10 Na_2So_4 solution were allowed to remain there for twenty minutes and were then transferred to sea water and fertilized. It was found in the course of about three hours that 27 out of 88 eggs had cleaved. The experiment was repeated and in this case the control showed two cleavages in 83 eggs while those treated with Na_2So_4 showed twenty normal embryos in 95 eggs.

Evidently, then, the antifertilization effect of the calcium ions on the egg may be reversed by sodium sulphate. Probably the $CaCl_2$ produces the gel condition in the egg membrane and the Na_2SO_4 redissolves the coagulum.

In order to test the hypotheses that the calcium or magnesium ions in sea water have the effect of slowly coagulating the membrane of the egg and of thus rendering it impermeable to its own sperm after a period of an hour or slightly more, the following experiment was undertaken :

Experiment VII. — A lot of eggs were stripped into distilled water, mixed thoroughly and then divided into five dishes containing respectively

A. Sea water.
B. $\frac{6}{10}$ *n.* KCl.
C. $\frac{6}{10}$ *n.* NaCl.
D. Equal parts of *B* and *C.*
E. $\frac{M}{10}$ Na_2SO_4.

After standing one and one-half hours a portion of the eggs from each dish was transferred to sea water, washed and an attempt made to fertilize, with the following result :

	Solution Used.	No. of Eggs Used.	No. of Embryos Formed.	Per Cent. of Embryos.
A.	Sea water.	78	0	0
B.	$\frac{6}{10}$ *n.* KCl.	45	14	31.1
C.	$\frac{6}{10}$ *n.* NaCl.	52	3	5.7
D.	Equal parts of *A* and *B.*	58	9	15.5
E.	M/10 Na_2SO_4.	63	42	66.6

After standing two and one-half hours another portion of the eggs was transferred from the original five dishes, washed and fertilized in sea water with the following result :

	Solution Used.	No. of Eggs Used.	No. of Embryos Formed.	Per Cent. of Embryos.
A.	Sea water.	68	0	0
B.	$\frac{6}{10}$ *n.* KCl.	51	8	15.7
C.	$\frac{6}{10}$ *n.* NaCl.	41	2	4.8
D.	Equal parts of *A* and *B.*	43	2	4.6
E.	M/10 Na_2SO_4.	58	14	24.1

After standing three and one-half hours the remainder of the eggs were transferred to sea water and fertilized, but in no case were any embryos formed.

The above series of experiments goes to show that the term of permeability of the egg membrane may be considerably prolonged by the exclusion of the calcium and magnesium salts. In no case, however, were the eggs capable of fertilization after standing for three and one half hours. It seems quite probable that the membrane is affected by internal changes in the protoplasm, which must be very active at this period. It is also to be noted that the $\frac{M}{10}Na_2SO_4$ solution helps the egg to retain its permeability longer than any of the ingredients of sea water. Further experimentation might lead to the discovery of a medium that would very greatly prolong the period of susceptibility to fertilization.

Conclusion. — These experiments show clearly enough that the membrane of the egg of *Fundulus heteroclitus* is subject to manipulation by the use of cations and anions, so that it becomes permeable or impermeable to its own sperm at the will of the investigator. I am unable to formulate any explanation of these changes in the condition of the membrane except that of a reversible coagulation and the consequent reversible surface tension.

When the surface tension of the egg is increased by coagulation of its colloids the sperm probably stands out on the surface of this altered membrane much as a minute droplet of water would stand out on the surface of an oil globule. Under ordinary conditions, however, the sperm surface and the egg surface are so adjusted to one another that they fuse on contact, and the sperm substance is engulfed by that of the egg.

We may suppose, then, that each species has a surface tension for the egg that is exactly suited to that of its own sperm and that, if altered, it becomes no longer suited to its own sperm but may become suited to other closely related sperm.

Loeb's results, cited at the beginning of this paper, offer evidence in favor of this view.

In the case of hermaphroditic animals, such as the tunicates with which Morgan worked, the condition is undoubtedly different. It seems probable that the egg and sperm of the same individual have a slightly incompatible surface tension of egg and sperm, an incompatibility that may be removed by the action of ether. It may also be readily understood why the sperm of one individual is not equally effective on the eggs of all other individuals, for some eggs have a surface tension more suitable to that of the sperm than others.

If in any way one could overcome the difficulties of surface tension and could inject the sperm of unrelated forms beyond the egg membrane without injuring the egg, we might get some very significant results. This is a field as yet unexplored but I hope not unpromising. These experiments will probably be continued with other eggs and over a more extended field.

Summary.

1. The calcium salts are the most active of the salts found in sea water in inhibiting fertilization. They are also the most active salts in precipitating colloids. Hence there is probably a causal relation between the precipitation of colloids and the inhibition of fertilization.

2. The seven salts $CaCl_2$, $BaCl_2$, $SrCl_2$, $MgCl_2$, $LiCl$, $NaCl$, and KCl inhibit fertilization in the order of their tension coefficients. Thus the egg is acted upon as if it were a colloidal system.

3. Supposing the egg to be a colloidal system with a membrane negatively charged it should be possible to precipitate or coagulate this membrane by salts of high positive tension coefficients, such as $CaCl_2$, and to redissolve this precipitate or coagulum by the use of salts of high negative tension coefficients, such as Na_2So_4. This was done successfully, fertilization being inhibited by $CaCl_2$ and restored by Na_2SO_4.

4. This alteration in the character of the membrane must involve an alteration in the surface tension that controls the entrance of the sperm.

5. The short term of susceptibility of the egg to fertilization by its own sperm, when the eggs are allowed to stand in sea water is probably due to the presence of calcium salts that slowly coagulate the membrane and thus increase its surface tension. The period of susceptibility to fertilization is considerably pro-

longed in the absence of calcium salts and especially in a medium of Na_2So_4.

6. These experiments and those of Loeb and Morgan seem to indicate that the relative surface tension of the egg and of the sperm is one important factor governing fertilization.

Marine Biological Laboratory,
Wood's Holl, Mass., August, 1905.

ON THE FOSSIL GENUS POROCYSTIS, CRAGIN.[1]

MAY M. JARVIS.

Under the name of *Porocystis pruniformis* F. W. Cragin described in 1892, in the Fourth Annual Report of the Geological Survey of Texas, curious spherical forms from the Cretaceous of Texas. In regard to the geological levels at which they were collected, he states: "In chalky limestones of the alternating beds in Travis, Burnet, Williamson, Lampasas and other counties, associated with Serpula, small Ostreidæ, and other forms indicative of moderate depth of water. In the Third Annual Report of the Survey, Mr. Taff has also recorded it from rocks of the Fredericksburg division of the Hickory-Cow Creek divide, Travis County, associated with several fossils of the *Exogyra Texana* beds."

Cragin considered that these fossils were Bryozoa, and speaks of having sent some to the late Dr. Ferdinand Roemer, who returned them marked "*? Parkeria*, sp. nov." His figures of them are not detailed, and for this reason as also because they are evidently not Bryozoa, a new description seems called for. There is a large series of specimens in the University Museum, the greater number considerably eroded but some with the surface sculpturation well preserved.

The tests are more or less prolate spheroids, sometimes one axis much shorter than the other. At one point on the surface is a flattened, very slightly protuberant area, free of any sculpturation, which might represent either the point of attachment of a stalk, or have been a large foramen. For various considerations it is probably the latter, so that the whole fossil proper would be a hollow spheroid with very thin shell and with a large opening at one end. The interior of each fossil is a mass of structureless limestone. This flattened area, or foramen as it may preferably be called, is usually upon the edge or very near it, when the specimen is much flattened, but in one of such cases it is in the center of the flat side. Canals radiate from the foramen, and the whole surface is covered with ridges and depressions.

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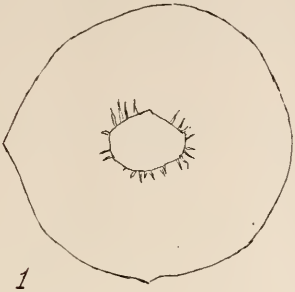
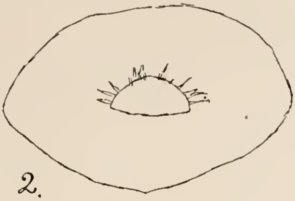
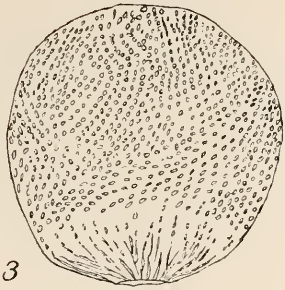
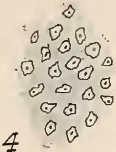
[1] Contributions from the Zoölogical Laboratory of the University of Texas, no. 70.

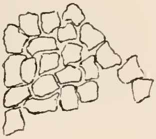
The usual surface sculpturation consists of rather irregular rows of approximately oval or circular depressions the centers of which are in some specimens raised into papillæ (Fig. 4). Some have flat scar-like white areas with traces of the more prevalent sculpturation (Fig. 5). Others have raised areas of the same











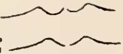


FIG. 1. Medium-sized specimen, showing general form, foramen and radiating canals.

FIG. 2. Specimen with one short axis, diameter 12 by 19 mm.

FIG. 3. Medium-sized specimen, diameter 18 by 18 mm.

FIGS. 4, 5. Different types of surface sculpturation.

FIG. 6. Ideal cross-section of test alone.

shape and arrangement ; these seem to indicate an erosion of the ridges and the papillæ. In some, again, the ridges have worn away, leaving only the papillæ, but the arrangement of these is as in the other cases. At the summit of each papilla, or at the center of each depressed area, is a minute foramen penetrating the wall of the test.

Fig. 7 shows the structure in ideal cross section. The whole interior is filled completely with foreign, calcareous substance, containing fragments of sponge spicules.

In no one specimen is the sculpturation preserved over the entire fossil, but a comparison of the different ones shows that the test in life, except just around the foramen, must have been so sculptured. The radial canals around the foramen are usually continued upwards as rows of minute depressions. Possibly such radial canals were not present in life, but produced by erosion of the exterior.

Measurements. — The largest specimens have a diameter of 25 × 31 mm. ; smaller ones from 9 to 18 mm. The large basal foramen measures from 4 to 7 mm. across.

These fossils present few good characteristics to enable one to decide what their nature was. But there is no resemblance, as Cragin had supposed, to Bryozoa, for even in a colonial ectoproctous Bryozoan the cysts of the separate individuals are more or less cylindrical, and there is no sign at all of any such structures. Evidently the structure is that of a very thin shell or test, without prolongations into the interior, with a large opening at one end and rows of numerous minute foramina elsewhere upon the surface. This general appearance suggests that it is a gigantic monothalamnian Foraminifer that in the course of fossilization has become infiltrated so as to be quite solid. Were it Bryozoan there should be present traces of skeletal parts within the test.

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